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Biology of the nematode *Mermis subnigrescens* Cobb

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BIOLOGY OF THE NEMATODE MERMIS SUBNIGRESCENS COBB

by

Melvin Walter Denner

**A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY**

Major Subject: Zoology (Parasitology)

Approved:

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Ames, Iowa**

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INTRODUCTION

Mermithids represent an unique family among the Nematoda. For many years, zoologists, particularly entomologists, have encountered long, threadlike, white to rusty-brown worms within various invertebrates, particularly arthropods. Limnologists have also reported finding such nematodes in the bottom fauna of various bodies of water. Most of these are known to be nematodes of the family Mermithidae Braun, 1883.

Their adaptations for a parasitic life include the lack of a functional intestine, the marked development of amphids and, in conjunction with them, a well-developed central nervous system in contrast to that of most parasitic nematodes. They differ as well from other nematodes in that they are parasitic only in the juvenile stages; adults are free living in water or in the soil.

Another interesting feature of mermithids is the sex ratio, for in many species females may exhibit male characteristics. This intersexuality may be shown in a series of individuals within a single species, ranging from normal females to those whose posterior body region appears obviously male, as evidenced by the presence of anal papillae and one or more spicules. In all reported cases, however, such intersexes are apparently functional females.

Sex determination usually occurs late in development and is apparently dependent upon the number of mermithids in each host, as shown by several workers (Christie, 1929, 1936, 1937; Cobb, Steiner, and Christie, 1927; Baylis, 1944). Although no investigations have been made on the relationship of size of host to number of parasites and their sex, the

host size may influence the sex ratio of mermithids harbored by an arthropod host. Numerous reports have shown that where the number of mermithids per host is low, females tend to predominate; where the number is high, males tend to prevail. In infections ranging between these extremes (e.g. from low to high), there is a gradient of sex ratio from female to male, respectively. This has been reported for at least four different genera and is probably typical of the family (Christie, 1929; Cobb et al., 1927; Johnson, 1955).

One important reason for the comparative obscurity of mermithids in the literature is the lack of knowledge relative to methods of their collection. As stated above, many aquatic species are inadvertently gathered in bottom faunal samples. Because mermithids resemble plant roots and usually move but slightly, they are frequently overlooked in such samples. A much more efficient method of recovering them is to examine possible hosts and to rear those which are infected until post-parasitic juveniles emerge. This procedure apparently has been followed only by Johnson (1955) in his studies on Hydromermis contorta, and by Phelps and DeFoliart (1964), the latter investigators having worked on Gastromermis viridis, Isomermis wisconsinensis and Mesomermis fluminalis. Most investigators appear to have discovered mermithids incidentally in the course of other investigations and hence have given only limited attention to them.

The data presented in this investigation result from researches carried out at the Iowa Lakeside Laboratory in northwest Iowa and at Iowa State University, Ames, Iowa.

Although life histories of some members of the family Mermithidae have been published, and some accounts of their morphology, physiology, pathology, and relationships to their hosts are available, Mermis subnigrescens Cobb, 1926, has not been extensively studied.

A collection of adult grasshoppers in the vicinity of Lake Okoboji during July and August of 1964 revealed the presence of a relatively high infection (approximately 17%) of mermithids. Subsequent collections of grasshoppers in the vicinity of Ames, Iowa, revealed a similar incidence of infections.

In attempting to identify these mermithids, it was found that only inadequate descriptions of the juvenile stages of M. subnigrescens existed. The present work will be devoted to a fuller account of juvenile stages of M. subnigrescens and, in addition, to a study of some pathological aspects, histochemical studies, electron microscopy, and bionomics of this parasite.

HISTORICAL REVIEW

The earliest mermithids known are fossils dating to the Jurassic Period (Taylor, 1935). Among the earliest was a species found by Heydon in 1861 in Rhine lignite. This nematode, named Mermis antiquua by Heydon (1861), was projecting from the posterior end of the beetle, Hesthesis immortua Heydon. Three specimens of another fossil mermithid, Mermis matutina, embedded in Baltic amber, were reported by Menge (1866) and were associated with an adult chironomid. The anterior end of one of these parasites protruded between two segments of the insect's abdomen. In 1935, Taylor reviewed fossil nematodes and suggested that these two species of mermithids be put into a new genus, Heydonius. He stated that it was impossible to tell in the first case if M. antiquua belonged to the genus Mermis. In the case of M. matutina, the presence of two spicules showed that it could not be assigned to the genus Mermis, which, according to Taylor, is characterized by possessing a single spicule. However, it is now well established that males of the genus Mermis invariably possess two spicules.

In 1842, Dujardin described a terrestrial mermithid, Mermis nigrescens and characterized the genus Mermis as follows: (1) body long and slender; (2) cuticle smooth but thickened and containing a layer of crossed fibers; (3) mouth terminal; (4) two lateral labial papillae; (5) four cephalic papillae posterior to the lateral papillae; (6) two lateral amphids; (7) vagina S-shaped; (8) two spicules in the male; and (9) six longitudinal muscle bundles divided by six longitudinal chords. In 1889, von Linstow

amended this description of the genus by specifying that: (1) spicules of the male are equal in length; (2) a thick cuticular esophagus is present; (3) the anus is lacking; (4) the vulva of the female is located approximately equatorial; (5) in the male, three to seven rows of papillae, mostly preanal, are found on the ventral side of the tail; and (6) a curved projection on the posterior end of the juvenile is present.

The first comprehensive work on Mermis was done by von Linstow in 1897. The genus Mermis at that time embraced all known mermithids. Filipjev and Schuurmans-Stekhoven (1941) separated the genus from other genera by limiting species of the genus to those possessing: (1) four cephalic papillae and four labial papillae; (2) a very thick cuticle; (3) eggs bearing filaments; and (4) males without copulatory bursae. Goodey (1963) described members of the genus Mermis as possessing: (1) a cuticle with criss-cross fibers; (2) terminal mouth with two lateral papillae close to it; (3) four head papillae placed further posteriorly; (4) two laterally placed amphids; (5) a S-shaped vagina; and (6) two posterior spicules in the male.

Von Siebold (1843) discovered an additional mermithid species which was designated as Mermis albicans. Schneider (1866), in his Monographie der Nematoden, recognized only two species of Mermis, namely M. albicans and M. nigrescens. He also described a third species, M. lacinulata in that year.

Von Linstow (1889) described a mermithid which he named Mermis contorta. His description was based on three females and a single male. Although the male presumably had left its host, the worm lacked any

evidence of spicules. He gave no suggestion as to the possible host and stated that he found the male worm in water near Göttingen, Germany.

At this same time, von Linstow (1889) separated the genus Paramermis from the genus Mermis, distinguishing the former from Mermis by the presence of a single spicule while those of Mermis possess two. He described two species in this group, Paramermis aquatilis and P. crassa.

In 1902, Corti described a third genus, Hydromermis. In the same year, de Man added another genus Pseudomermis. Since 1900, approximately twenty-five different genera of mermithids have been described.

Descriptions of many mermithids have been based sometimes on a single specimen, or, more frequently, on less than five immature specimens. An extreme example of this occurred when Steiner (1929) erected Eumermis behningi on the basis of several pieces of what appeared to him to be a single female specimen obtained from a river pool of the Volga.

Steiner (1929) described several other new species on the basis of a single juvenile specimen and, in at least one case, admitted that the specimen was in poor condition. Other examples of investigators who erected new species on the basis of fewer than five adults and/or post-parasitic juveniles include Baylis (1944), Micoletzky (1923), von Linstow (1899), and Coman (1955). With the exception of possibly twelve species of Mermithidae, descriptions of all other mermithids appear to be based on fewer than ten worms. Descriptions of mermithids are frequently based on postparasitic juveniles or on the adult with no information presented as to the host. To date, very few comprehensive studies on the parasitic stages of these nematodes have appeared, other than the recent works of

Johnson (1955) and Phelps and DeFoliart (1964). Christie (1936) described the life history of Agamermis decaudata and included a detailed description of various stages of the juvenile development.

Polozhentsev (1953) listed eighteen genera in addition to four questionable genera of Mermithidae. Goodey (1963) listed the names of thirty different genera including one fossil species. It is apparent from the foregoing discussion that investigators differ greatly in their opinions relative to the validity of genera belonging to the family.

Many investigators comment on the economic importance of mermithids, especially their role in the biological control of economically important insects and insect pests. The United States Bureau of Plant Industry has used A. decaudata and M. subnigrescens in an effort to control grasshopper populations (Christie, 1936). The government has also used members of this family to control, in selected areas, the mosquito populations in the Arctic. Japanese investigators have reported that mermithids, parasitic in the rice borer, help to keep this pest within bounds (Sugiyama, 1956a, 1956b; Imamura, 1932; Kaburaki and Imamura, 1932).

Chittenden (1905) reported an interesting adverse economic consequence of mermithids. During the early 1900's, newspapers carried reports that mermithids were the cause of deaths of humans having ingested them with cabbage. One report stated that a "State Chemist" had examined the worm and reported that it contained enough poison "to kill eight persons". In Raleigh County, West Virginia, the cabbage crop was a complete failure and it was reported that "...there was enough poison contained in one worm to poison 25 men." (Chittenden, 1905). None of these reports had any founda-

lion in fact. Chittenden further reported that the fear of poisoning spread from West Virginia to Virginia, Tennessee, South Carolina, Louisiana, into Florida and in addition westward to Kentucky, Illinois, Iowa, Missouri, Oklahoma, and Colorado. In parts of Illinois the fears of growers and purchasers were such that farmers were letting their cabbage go to waste. At Quinter, Kansas, "...quantities of cabbage shipped to Colorado were reported burned because of the presence of the hair-worm"¹ (Chittenden, 1905). In Tennessee in 1904, fully 85% of the cabbage crop of the state was lost. Finally, in Missouri, hundreds of barrels of sauerkraut were destroyed through fear that "...the dreaded snake might be part of the ingredients". Many gardeners claimed that they could not sell a single head of cabbage on account of the "snake scare" (Chittenden, 1905).

Some investigators (Callot, 1959; Callot and Kremer, 1963; Wülker, 1961; Rempel, 1940; and Wheeler, 1900, 1907, 1928, and 1929) have shown that mermithids may influence the sex development of the host. In Culicoides albicans (Callot, 1959), and in Chironomus rempeler (Thieneman, 1954), the time of entrance and the number of mermithids in the larva determine the degree of intersexuality in the adults. Similar examples are cited by Wheeler in various ant species (Wheeler, 1928). Thus, there are ample biological and economic reasons for studying this family.

Apparently the first reported incidence of a mermithid from a grasshopper was that by von Siebold in 1848. He reported Mermis albicans from

¹"Hair-worm" here apparently refers to mermithid worms and not to Nematomorpha.

Stauroderus biguttulus (L.). In 1850, von Siebold described a new species, Mermis barbitistides-autumnalis, from Barbitistes autumnalis Brulle and in the same year he described Mermis dectici-brevipennis Diesing from Chelidoptera roesellii Hagenbar.

Diesing (1851) listed the following species of Mermis and their orthopteran hosts known at that time:

Species	Hosts
<u>Mermis nigrescens</u> Dujardin	<u>Gomphocercus morio</u>
" "	<u>G. parapleurus</u>
" "	<u>Decticus verrucivorus</u>
" "	<u>Locusta viridissima</u>
<u>Mermis albicans</u> Siebold	<u>Oedypoda stridula</u>
<u>Mermis blattae orientalis</u> Diesing	<u>Blattae orientalis</u>
<u>Mermis barbitistides autumnalis</u> Siebold	<u>Barbitistes autumnalis</u>
<u>Mermis dectici brevipennis</u> Siebold	<u>Decticus brevipennis</u>
<u>Mermis gomphoceri viriduli</u> Siebold	<u>Gomphocercus viridulus</u>
<u>Mermis notodontae dromedarii</u> Siebold	<u>Notodontae dromedarii</u>
<u>Mermis micryphantidis bicuspidati</u> Siebold	<u>Micryphantes bicuspidatus</u>
<u>Mermis mantidis-religosae</u> Diesing	<u>Mantis religiosa</u>

A list of reported North American orthopteran hosts parasitized by mermithids is included in the Appendix.

The first report in the United States of mermithids occurring in Orthoptera appears to be that of Leidy (1851). He reported having found Mermis albicans in Locusta carolina (L.). In 1856, Leidy reported Mermis

albicans from Orchelium gracile Harris and from Dissosteira carolina (L.). However, it should be pointed out that Cobb et al. (1923) remarked that "...as far as can be judged, the European albicans as described, has not yet been found in this country (U.S.A.)."

The two most common mermithids parasitizing Orthoptera in the United States appear to be Mermis subnigrescens Cobb and Agamermis decaudata Cobb, Steiner, and Christie.

MATERIAL AND METHODS

A preliminary survey in 1964 indicated that the grassy areas of Iowa Lakeside Laboratory and four areas in and near Ames, Iowa, had large populations of grasshoppers and a high incidence of mermithid infection, including M. subnigrescens. The five areas and their locations are as follows:

1. A small area adjacent to an apple orchard. Major plants in the area include white and red clover, dandelion, Canadian thistle, and little bluestem grass. Location: R-24W-T84N-32.
2. The I.S.U. arboretum, an area of rolling hills adjacent to a small creek. Several species of trees are present. The region is mostly grass covered. Location: R-24W-T83N-17.
3. Vacant lot north of the Northside Fruit and Grocery parking lot in Ames. The area, mowed twice yearly, consists of flat hayland. Major plant cover consists of yellow, white, and red clover, Canadian thistle, and big and little bluestem grass. Location: R-24W-T84N-5.
4. A farm field planted to clover in 1965, to corn in 1966, and to soy beans in 1967. Location: R-25W-T83N-4.
5. Iowa Lakeside Laboratory, Lake Okoboji in Northwest Iowa. Location: R-37W-T99N-22.

In addition, gravid females of Mermis subnigrescens were collected from the following areas:

1. An abandoned railroad right of way. Heavy plant cover consisted mainly of various grasses, many trees, and wild flowering plants. Loca-

tion: R-24W-T83N-21.

2. A county road ditch. A heavy grass cover consisted of big and little bluestem grass. Location: R-25W-T83N-4.
3. The grassy area adjacent to the apple orchard noted above.

In addition to the above areas, one collection of grasshoppers was made on a farm pasture one mile west of Bardstown, Kentucky.

Field collections of grasshoppers were made by using a heavy duty insect sweeping bag. Insects from the sweeping bag were transferred to an empty coffee can modified by placing a nylon stocking, open at both ends, over the open end of the can.

Insects were brought back to the laboratory and placed in a refrigerator at 0° C. for approximately ten minutes or until they became quiescent. They were then removed and the grasshoppers were separated from the other insects and plant material.

Some collections of grasshoppers were placed in a rearing cage measuring 3' x 3' x 3'. Sides of the cage were of wire screening; the top and bottom were of ½" plywood. The top was provided with a 3' x 1.5' swing out door. The bottom of the cage was covered with soil varying in type from black topsoil at one end of the cage, followed by an area provided with a mixture of equal parts of topsoil and sand, to sand alone at the opposite end of the cage. Since several species of grasshoppers were maintained in the cage, this soil gradient satisfied the individual preferences for egg laying for each species. Soil was kept moist, but not so wet so as to become packed. The cage was checked daily and dead grasshoppers were removed and dissected for parasites. Some mermithids

obtained in this manner were kept in plastic containers containing moist soil and provided with a tightly fitting plastic bag over the top. All remaining parasites were killed and preserved for later use. The rearing cage also afforded a convenient method for collecting large numbers of postparasitic juveniles. These entered the soil and remained there until they were removed for observation.

Most grasshoppers were dissected shortly after having been collected in nature. After the ten-minute period of refrigeration, grasshoppers were placed in a stoppered 500 ml Erlenmeyer flask to which a wad of cotton saturated with ethyl ether had been added. Grasshoppers died within several minutes and were then keyed to family and, if possible to genus and species by use of the key by Froeschner (1954). Information for each collection included the following: date, location, total number of grasshoppers collected, number infected, total number of parasites, host, number of parasites per host, and number of parasites recovered per taxonomic category of the host. Most dissections were done by use of microdissection equipment.

Several naturally infected grasshoppers were utilized solely for histological investigations. After killing or anesthetizing these insects, a small incision was made in the pleura between segments four and seven. This provided a rapid method for determining the presence of mermithids in the haemocoel with minimal host-tissue disruption, and also allowed fixatives to surround the internal organs. Parasitized and normal grasshoppers were fixed in Bouin's fixative at approximately 24° C. After standing for three to five days, specimens were washed in several

changes of 50% ethyl alcohol, one change of 70% alcohol containing one per cent potassium acetate, and dehydrated according to the Martignoni (1960) methyl benzoate method. Infiltration was done with 56-58° C. Paraplast (Fisher Scientific Co.) in a vacuum paraffin oven set at 60° C. Other specimens were doubly infiltrated, i.e., after dehydration, specimens were left for two days in a four per cent celloidin solution, then were hardened in chloroform, and infiltrated in equal volumes of chloroform and Paraplast. Further infiltration was provided by two changes of paraffin for two hours each, and by final infiltration in the vacuum paraffin oven. In either case, embedding was done in 56-58° C. Paraplast. Specimens were sectioned at eight and ten microns. Stains used included Heidenhain's iron haematoxylin, eosin, and Mallory's triple stain for connective tissue. Experimentally infected grasshoppers were treated similarly.

Fixation of all stages of the parasite was accomplished by using Bouin's, buffered ten per cent formalin (pH 6.8), Baker's formalin (pH 6.5), Helly's, Susa's, Zenker's, Carnoy's, Schaudinn's, and Kahle's fixatives. Helly's and Zenker's gave superior results for Mallory's triple stain.

Parasites selected for whole mount study were killed in hot 70% alcohol plus ten per cent glycerol. Specimens were allowed to clear in this mixture, during which time the alcohol was allowed to slowly evaporate. Some specimens were also cleared in lactophenol, but this cleared the worm excessively. In many cases, by use of either method, it was necessary to tint the worms with Grenacher's borax carmine, a one per cent acid

fuchsin solution, a one per cent methyl blue solution, or a one per cent aniline blue solution. Whole worms were mounted in glycerine jelly and the cover glass was sealed with fingernail polish. Alternately, the double coverglass method was used for mounting.

Parasites were prepared for use with the International Cryostat CT I either by fixation in ten per cent buffered formalin or by use of untreated fresh tissue. Nematodes, cut in 10-15 mm lengths, were transferred to a 20% aqueous solution of commercial gelatin warmed to about 40° C. Specimens were incubated in this solution for three hours after which time the solution was allowed to cool and solidify. Blocks of solidified gelatin containing the specimens were hardened in ten per cent formalin for 18-24 hours. Fresh material was handled in the same manner except that it was incubated for only 15 minutes.

A second useful procedure for cutting sections for the cryostat consisted of transferring the pieces of tissue to a mixture of equal volumes of gum mucilage (50 g gum acacia in 50 ml distilled water with the addition of a crystal of thymol or phenol) and sugar syrup (50 parts, by volume, of cane sugar dissolved in 25 parts of water). Pieces of tissue were kept in the above mixture for approximately 24 hours. Sections could be cut at two to four microns by use of these methods.

Sections of tissue embedded in gelatin were placed on glass slides and warm air dried. Any gelatin adhering to the tissue after sectioning was removed by carefully running a slow stream of warm water over the slide. The slide was again dried by warm air and stained. Sections of tissue embedded in the gum and syrup mixture were affixed to slides by

warm air drying and were then stained.

Grasshopper eggs, collected from soil in the rearing cage, were retained for subsequent use by moistening and placing them in a refrigerator set at -10° C. for one day and then transferring them to refrigeration at 5° C. for three or more weeks. Eggs were hatched in an aquarium covered with a glass plate. A desk lamp, equipped with a 100-watt bulb, was placed on the glass plate in such a manner that the bulb touched the plate so that the resultant heat was sufficient to break diapause.

Gravid female M. subnigrescens were collected from the field during and immediately after a rain. Generally, at least a quarter of an inch of rain had to fall within an hour to cause emergence of the worm from the soil. More worms seemed to emerge when a light shower continued over a period of several hours with a total accumulation of at least one-half inch of moisture.

The worms, found crawling on vegetation, were brought back to the laboratory and placed on moist filter paper. By confining the worm to a small area on the filter paper, numerous eggs were laid in a small area. When sufficient numbers of eggs were laid, or the female ceased laying them, the worm was returned to moist soil in the laboratory and kept there until needed again. In this manner, the worm could be induced to lay large numbers of eggs several times in a period of approximately three months.

Grasshoppers were exposed to the eggs of M. subnigrescens by removing a number of eggs from the filter paper with a camel's-hair brush and coating a piece of lettuce leaf with the eggs. Grasshoppers, starved for 24 hours prior to the exposure, were allowed to feed at will on this lettuce.

When individual infections with known numbers of eggs were desired, the preferred method was as follows. After oatmeal was moistened with hot water to a consistency of thick paste, a specified number of eggs could be placed in a small pellet of the oatmeal and fed to individual grasshoppers. This method is slow and tedious, and it is not recommended when large numbers of grasshoppers are to be exposed, in which case, the lettuce method is preferred.

Laboratory reared grasshoppers were injected with mermithid exudate and an extract of mermithid worms to determine the effect on host reproductive processes. To prepare the exudate, five M. subnigrescens females were placed in 35 ml Ringer's insect solution modified by adding 0.5% glucose and 0.1% trehalose for four days. Prior to injection, the exudate without the worms, was heated to 85°C and cooled. Worm extract was made by homogenizing the entire worm in a glass homogenizer fitted with a ground glass pestle. Twenty milliliters of ethyl ether with a drop of acetic acid were added to the worms during homogenation. The ether was allowed to evaporate completely over a period of several days. The extract was then reconstituted by adding 40 ml of the modified Ringer's insect solution.

For handling grasshoppers during the injection, a particularly good method involved placing twenty grasshoppers in a 500-ml Erlenmeyer flask stoppered with a wad of cotton. The flask with the grasshoppers was placed in a tub of cracked ice until they became quiescent. A glass plate placed on cracked ice was used to support quiescent grasshoppers while they were being injected. Injections were made into the pleura

between segments seven and eight, the needle being directed anteriad.

Postparasitic juveniles of Mermis subnigrescens were selected for examination by electron microscopy and were cut in 1-mm length pieces before fixation in phosphate buffered three per cent glutaraldehyde. Phosphate buffered (pH 7.2) sucrose was used to rinse the tissues prior to post-fixation in one per cent osmium tetroxide. Tissue was then dehydrated in an ethanol series and embedded in a 1:1 mixture of epon-araldite. Ultrathin sections were cut on a Porter-Blum MT-2 microtome and mounted on carboned Parlodion coated grids. Sections were stained in two per cent uranyl acetate for 15 minutes, then rinsed and stained further in lead citrate solution for 15 seconds according to the method of Venable and Coggeshall (1965). Prepared grids were viewed using a RCA-EMU II microscope.

SUMMARY OF LIFE CYCLE

The life cycle of Mermis subnigrescens involves a single orthopteran host. In early summer, embryonated eggs of this species, containing a second stage juvenile, are deposited on vegetation by gravid female worms. Eggs ingested by members of the Family Acrididae (short-horned grasshoppers) or Family Tettigoniidae (long-horned grasshoppers) hatch in the anterior portion of the alimentary canal. Parasitic juveniles penetrate the intestinal wall, enter the haemocoel, and continue development. A second moult of the parasitic juvenile apparently occurs between the ninth to eleventh day after ingestion by the host.

Male juveniles usually remain in the host from four to six weeks, attaining a length of from 20 to 60 mm, and female juveniles usually remain in the host from eight to ten weeks attaining a length of 50 to 135 mm.

In late summer, parasitic juveniles emerge from the haemocoel through the body wall of the host. Postparasitic juveniles then enter the soil and undergo a third moult approximately two to four months later. Female postparasitic juveniles attain sexual maturity in the soil approximately eight months after emergence from their host. The following spring, females may mate with males and begin egg production, or they may produce eggs parthenogenetically. Egg production continues for a year but few, if any, eggs are laid before the end of this time, i.e., approximately 20 months postemergence. The gravid female lays eggs on vegetation, usually during or immediately after a rain.

Males of M. subnigrescens reach sexual maturity shortly after the postparasitic moult. The life span of members of this species is approximately two and one-half years.

BIONOMICS

Eggs

Unlike another orthopteran mermithid (Agamermis decaudata), eggs of M. subnigrescens are not deposited in the soil, but are laid on stems and leaves of vegetation. This occurs during or immediately following rain. When rains cease and foliage dries, females coil up, fall to the surface of the ground, and enter the soil.

Christie (1937) stated that a gravid female of Mermis subnigrescens 85 mm long contains approximately 14,000 eggs. The present studies tend to agree with this.

The eggs are subspherical, somewhat compressed at the poles, and measure approximately 48-54 microns from pole to pole and 50-56 microns at the equator. Two protective coverings surround the egg. The outer, dark-brown covering, which may easily be broken and removed, has a fracture line across the equator (Figure 1). A second covering consists of a light-brown membrane lying beneath the outer covering and is approximately 2.5 microns in thickness (Figure 2).

Each hemisphere of the outer covering has a thickened area at the pole formed by the attachment of two many-branched, albuminous appendages or byssi (Christie, 1937). These apparently assist in attachment of the eggs to various surfaces and may also keep them together in small clumps.

At oviposition, each viable egg contains an infective second-stage juvenile, according to Christie (1937). In my studies, no evidence of moulting within the egg was observed. Eggs may remain viable throughout

the summer on foliage, their dark color presumably shielding the juvenile from harmful sun rays. Some eggs were kept in the laboratory on moist filter paper and these retained their viability for approximately six months.

In 1967, maximum egg laying occurred during the second and third week of June. The first gravid females were found on May 22; the last gravid female was found the last week of June. Christie (1937) has found similar results and reported that at Woods Hole, Massachusetts, the time of maximum egg deposition was usually during June and July, and that egg laying was controlled by weather conditions. In the present study, there were only light rains during July and the first part of August, 1967. These rains were apparently too light to bring mermithids to the surface. Rains in the latter part of August, however, were considered heavy enough, but no gravid female worms were found.

Gravid females were kept in the laboratory for use as a constant source of eggs, and could be stimulated to lay eggs several times over a period of two to three months (May to July, 1967). All gravid females died by the early part of October, 1967, and no eggs could be procured after the end of August. This would seem to indicate that following egg laying, the spent female may live less than two months. Very few eggs remain in the uterus of spent females, and cross sections of these worms show the trophosome to be nearly empty of spheres (Figure 2).

Observations of laboratory reared mermithids seem to indicate that egg development takes place by the eighth month after emergence of the juvenile from the host. Dark spheres appear in the area of the ovary by the sixth

month, but it could not be determined if these were unembryonated eggs or if they were trophosomal spheres, since both were approximately the same size. Approximately 100-150 eggs were estimated to be present in the reproductive tract by the end of the eighth month. Once gravid, these female worms, raised in the absence of males, were induced to produce eggs, parthenogenetically, by placing them on moist filter paper. A series of eleven monthly attempts have been made without a single egg being laid, but two of three females used for this purpose have died.

Because of lack of sufficient experimentally reared males, no data could be gathered as to the time needed for the attainment of sexual maturity. However, they apparently become sexually mature shortly after the first postparasitic moult.

Cobb (1926b) described a type of chromotropism associated with the egg laying of M. subnigrescens. According to his studies, release of eggs is due to the accumulation of the pigment haemoglobin in the anterior end and, to a lesser degree, throughout the body of the female. The presence of this pigment, in sunlight, provides the stimulus for egg laying as shown by Cobb (1926b) and later confirmed by Christie (1937). However, Ellenby and Smith (1966a), working on haemoglobin pigmentation in M. subnigrescens, suggest that the distribution of pigment is more closely associated with respiratory functions and is particularly related to oxygen supply. They concluded that the concentration of pigment is not delimited enough to function in chromotropism. The present study seems to indicate that although sunlight is not necessary for egg laying, it may influence it to some extent. This was evidenced by the somewhat greater

number of eggs laid in the presence of sunlight than in darkness.

Numerous examples of other nematodes possessing haemoglobin may be cited and ample evidence exists that haemoglobin serves as a source for oxygen in nematodes (von Brand, 1966; Ellenby and Smith, 1966a, 1966b).

On two occasions during the summer of 1967, female Mermis subnigrescens were found climbing on grass at night after an evening rain. These worms were found by using a flashlight. The leaves and stems with the worms were taken back to the laboratory and examined for eggs, which were found in abundance in the vegetation. Several of these gravid females were placed on individual pieces of moist filter paper which were then placed in covered containers over night in a dark room. When these pieces of filter paper were examined the next day, from 500-1500 eggs were present on each piece of filter paper. This is convincing evidence that oviposition may occur in the absence of sunlight.

Two attempts were made in late May to collect gravid females at night after a rain, but these attempts were unsuccessful. Only after the middle of June were gravid females found at night. This may indicate that temperature, rather than sunlight, is an important stimulus for egg laying.

Natural Infections

Christie (1937) examined 2,500 grasshoppers from New Hampshire and found an average infection of mermithids of 12% but did not identify them other than reporting that of 66 collections, 42 were exclusively M. subnigrescens, 17 collections consisted of both M. subnigrescens and

Agamermis decaudata, six collections consisted of only A. decaudata, and one collection of Hexamermis sp. only. Blickenstaff and Sharifullah (1962) found 25% parasitism by mermithids in Missouri. In Quebec, 12 to 23% infection by M. subnigrescens was recorded by Briand and Rivard (1964). Smith (1940), in a ten-year survey of mermithids near Belleville, Ontario, found a three per cent annual average and a maximum of 32% in one area. In 1958, Smith recorded a progressive decrease of mermithid parasitism in grasshoppers from eastern to western Canada, and an average of 10% in infected areas. Hayes and DeCousey (1938) reported a 14% infection of grasshoppers, mostly Melanoplus femurrubrum and M. differentialis, near Urbana, Illinois in 1937.

In the present study, collections of grasshoppers made in Iowa during 1965-1967 involved the dissection and examination of 6,872 orthopterans. Table 1 is a comparison of infections as determined in this study for Iowa.

Table 1. Annual infection of grasshoppers by mermithids in Iowa for the years 1965-1967

Year	Number of grasshoppers		Per cent infection
	Dissected	Infected	
1965	787	140	17.7
1966	1803	190	10.5
1967	4282	819	19.1
Total	6872	1149	16.7

From the above table, it appears that the average infection of the grasshopper population in Iowa is approximately 16-17%. The particularly low percentage of infection in 1966 can be attributed in part to the exceptionally dry months of June, July, and August of that year. Relative lack of rain during these months probably prevented deposition of eggs in appreciable numbers. Christie (1937) stated that M. subnigrescens females which were prevented from coming to the surface to deposit eggs, would remain in good condition for several years. He further reported that such females were gravid and had apparently laid few eggs. The rather high percentage of infection in 1967 then, would tend to indicate that those females which were prevented from laying eggs in 1966 probably laid their eggs in 1967. Since two years are generally required for females of M. subnigrescens to become gravid, these eggs laid in 1967 were probably from females having emerged from their hosts in 1964 and 1965. This would then cause an unusually high number of eggs to be present on vegetation in a normal year and would result in a higher incidence of infection. The relatively high rate of infection of 1967 suggests that this indeed is what occurred.

Sex of the host and its ability to harbor infection shows considerable variability. Glaser and Wilcox (1918) found mermithid parasites in about 45% of female grasshoppers but only about nine per cent of the males. Christie (1936) found that 10.7% of male Acrididae (short-horned grasshoppers) and 10.2% of the females were infected with Agamermis decaudata. In the family Tettigoniidae (long-horned grasshoppers), he found 19.6% of the males and 14.6% of the females parasitized with A. decaudata.

Table 2 is a compilation of results of sex ratios of infected grasshoppers in this study for the years 1965-1967 in Iowa.

Results from Table 2 seem to indicate that there is a slightly higher percentage of females infected with mermithids. The unusually high incidence of mermithid parasitism in females hosts reported by Glaser and Wilcox (1918) is difficult to explain and they offered no explanation. The incidence records of Christie (1936) appear to be the more normal situation.

Table 2. A comparison of infection by mermithids of male and female grasshoppers in Iowa for the years 1965-1967

Year	Number of grasshoppers				Per cent infected	
	Collected		Infected			
	Male	Female	Male	Female	Male	Female
1965	361	426	66	74	18.1	17.3
1966	864	939	91	99	10.5	10.5
1967	2229	2053	390	429	17.5	20.9
Totals	3454	3418	574	602	15.8	17.6
					(Average)	

Three subfamilies of orthopteran hosts (Cyrtacanthacridinae, Oedipodinae, and Conocephalinae) were the principal sources of data studied with reference to natural infection of mermithids. Table 3 is a comparison of these subfamilies. The subfamilies Cyrtacanthacridinae and Oedipodinae belong to the family Acrididae; the subfamily Conocephalinae, to the family Tettigoniidae.

Table 3. A comparison of mermithid infection in selected subfamilies of Orthoptera

Orthopteran hosts	Total no. examined	Per cent of total Orthoptera	Per cent infected
Family Acrididae			
Cyrtacanthacridinae	2414	35.2	16.9
Oedipodinae	1548	22.6	14.9
Family Tettigoniidae			
Conocephalinae	392	5.7	17.6
Other families of Orthoptera	2518	36.5	17.1

As indicated in Table 3, it appears that the rate of infection with mermithids is approximately equal in the three subfamilies. Mermis subnigrescens was found only in the two families Acrididae and Tettigoniidae. Mermithids, later identified as Agamermis decaudata were found in the family Gryllidae. Attempts to experimentally infect individuals of the family Gryllidae (Acheta assimilis F. and Nemobius sp. Serv.) with M. subnigrescens proved unsuccessful in this study.

An attempt was made to determine the relative abundance of both Mermis subnigrescens and Agamermis decaudata having emerged from naturally infected orthopteran hosts maintained in the laboratory. Specific identification of juveniles is difficult because this stage lacks morphological structures which can be used to separate the two species. This is especially so when juveniles are less than several weeks old. Following the escape from their hosts, however, postparasitic juveniles may be identified with less difficulty. It could then be determined that ap-

proximately 5.8 to 6.0% of over 800 grasshoppers examined were A. decaudata and the remainder M. subnigrescens.

It would appear that A. decaudata contributes only a relatively small percentage to the total grasshopper infection. The situation is complicated by the fact, as reported by Christie (1936), that usually only two to three A. decaudata occur in each host while M. subnigrescens may be found in large numbers per host. Christie reported that grasshoppers may harbor 20, 30, or, on rare occasions, over 100 M. subnigrescens juveniles. Therefore, the true rate of infection of A. decaudata in grasshoppers is probably greater than the 5.8 to 6.0% indicated above.

Experimental Infections

Christie (1937) recorded a number of species of grasshoppers which were naturally and/or experimentally infected with M. subnigrescens. Experimental infections occurred in Arphia sulphurea (F.) and Romalea microptera (Beauvois). Naturally infected hosts included Chortophaga virifasciata (DeG.), Conocephalus brevipennis (Scudder), Encoptolophus sordidus (Burm.), Melanoplus bivittatus (Say), M. mexicanus (Saussure) and Orphulella pelidna (Burm.). Natural and experimental infections were found in Camnula pelucida (Scudder), Chorthippus longicornis (Latreille) and Melanoplus femurrubrum (DeG.).

A listing of naturally and/or experimentally infected species in this study is given in Table 4. In experimental infections, hosts were examined 28 days after exposure to Mermis subnigrescens eggs. All feeding experiments involved the coating of lettuce with varying numbers of

Table 4. Host-list of experimental and/or natural infections of
M. subnigrescens

Species	Type infection N = Natural E = experimental
<u>Chorthippus longicornis</u> (Latreille)	N
<u>Chortophaga viridifasciata</u> (DeGeer)	N, E
<u>Conocephalus strictus</u> (Scudder)	N
<u>Conocephalus</u> sp. (probably <u>fasciatus</u>)	N
<u>Dissosteira carolina</u> (Linnaeus)	N
<u>Encoptolophus sordidus sordidus</u> (Burmeister)	N, E
<u>Melanoplus bivittatus</u> (Say)	N, E
<u>M. differentialis</u> (Thomas)	N, E
<u>M. femurrubrum</u> (DeGeer)	N, E
<u>M. mexicanus mexicanus</u> (Saussure)	N, E
<u>Neoconocephalus ensiger</u> (Harris)	N
<u>Orchelimum</u> sp.	N
<u>Phoetalictes nebrascensis</u> (Thomas)	N
<u>Schistocerca americana</u> (Drury)	N
<u>Scudderia furcata furcata</u> (Brunner)	N

viable eggs and allowing the grasshoppers to feed naturally.

An experiment was carried out to determine if a grasshopper once infected with Mermis subnigrescens is refractile to additional infections. Twenty Melanoplus spp. (mostly M. femurrubrum) consisting of 13 females and 7 males were selected. One of the females died during the last half

of the experiment. For this experiment, grasshoppers were each fed 5-10 eggs of M. subnigrescens placed on lettuce at three separate times, namely July 7, July 20, and August 1, 1967. On August 7, these grasshoppers were killed, dissected and examined for evidence of mermithid juveniles. Results are given in Table 5.

As can be seen from Table 5, there appears to be a reduction in number of juveniles resulting from the second and third exposures. In one grasshopper, a male, no juvenile stage resulting from the second feeding was recovered. All other grasshoppers possessed all three stages of juveniles. It can therefore be assumed that throughout the life of the grasshopper, multiple exposure to mermithid eggs results in the grasshopper's acquisition of numerous parasites of varying age, but that initial infections tend to be heavier.

Table 5. Susceptibility of grasshoppers (Melanoplus spp.) to reinfection with M. subnigrescens

No. hosts infected	Average number juveniles/host exposure		
	7/7/67	7/20/67	8/1/67
12 females	4.10	2.75	2.9
7 males	3.14	2.00	2.3

Juveniles

Growth rate and developmental stages

Development of juvenile Mermis subnigrescens was studied using a series of worms obtained from experimentally infected grasshoppers. All

observations noted below are based on studies of living and on unstained fixed parasites from grasshoppers (Melanoplus spp.) between four and five weeks old each of which had been fed five to ten eggs of Mermis subnigrescens. The number of juveniles used in measurements of length and width varied from one to fourteen individuals and are summarized in Table 6, together with measurements presented by Baylis (1944) for parasitic juveniles of M. nigrescens. Although sex could not be precisely determined in the parasitic juveniles of M. subnigrescens, it was assumed that if a host harbored four or fewer parasites, these juveniles were all females. Christie (1929) reported that if a host harbored fewer than five or six mermithids, all juveniles invariably were females.

Eggs of Mermis subnigrescens do not hatch until they are swallowed by a grasshopper or, in some cases, by other phytophagous invertebrates. In the present study, eggs hatched in the alimentary canal within one and one-half to three hours after ingestion by 26- to 32-day-old grasshopper nymphs of various species. Most juveniles apparently hatch in the posterior region of the ventriculus and in the anterior portion of the ileum. Juveniles penetrate the wall of the alimentary canal and enter the haemocoel where they continue their development (Figure 3). Newly emerged parasitic juveniles are approximately 180 microns long and ten microns in width.

According to Christie (1937), the recently hatched juvenile of M. subnigrescens measures 740 microns long by 34 microns wide. However, his Figure 3 shows this juvenile to be approximately 340 microns by 20 microns. Baylis (1944), working with Mermis nigrescens, found that

Table 6. Comparative measurements (in millimeters) of parasitic female juveniles of M. subnigrescens and M. nigrescens

Day	No. of hosts	<u>Mermis subnigrescens</u>			<u>Mermis nigrescens</u> Baylis (1944)	
		No. of specimens per host	Average length	Average width	Average length	Average width
1	3	1,2	0.18	0.010	0.24	0.010
2					0.20	0.010
3	1	1	0.23	0.010		
5	2	1,2	0.34	0.018		
6	3	4,4,4	0.40	0.030		
7	1	2	0.79	0.040	0.20	0.014
8	2	2,3	1.03	0.054		
9	3	3,3,4	1.18	0.056	0.30	0.025
11	4	4,4,6	2.67	0.080	1.20	0.060
12	1	3	5.01	0.100	1.35	0.065
13	1	4	7.50	0.120	1.50	0.070
14	1	1	9.90	0.130		
15	2	4,3	12.20	0.140		
16	4	1,2,2,3	27.00	0.160		
18-22					5.60	
28	5	3,3,3,4	44.30			
31-34					25.00	
34	1	5	68.00			
36	1	3	76.00			
37	1	3	78.00			
39	2	2,5	93.00			

juveniles, newly liberated from the eggs by cover glass pressure, measure approximately 240 microns and that they are ten microns in maximum width. The unusually long length of 740 microns as reported by Christie is clearly an error. The scale used in his Figure 3 must also be in error. Otherwise the "recently hatched larva" designation accompanying his Figure 3 is a misrepresentation as to the actual age of the juvenile. Certainly the measurements that Baylis gives for the recently hatched Mermis nigrescens are more accurate.

The fusiform body of newly hatched juveniles is bluntly squared anteriorly and bluntly rounded posteriorly. The extremities of the living or unstained fixed worm appear clear; the remainder of the body appears as an amorphous, granular mass. The only obvious structure is the anterior stylet (onchium of Cobb, 1926a) approximately ten to twelve microns long. The stylet can be extruded for about half its length (Figure 4). Several rows of small cells of undetermined nature and extending the length of the worm appear in stained sections (Figure 3), but these are not apparent in living or in unstained fixed whole mounted specimens.

No two-day juveniles were observed. However, in a single juvenile three days old, measuring 230 microns long and ten microns wide, little further development has occurred. There appears to be some cellular arrangement within the juvenile, but no definite structures could be observed. The stylet shows no marked change from that of the first day juvenile.

In juveniles five days old, body length has increased to an average of 340 microns and body width to 18 microns. Further organization of

structures is apparent for anterior and posterior ends show masses of undifferentiated cells. Posteriorly, these cells are somewhat obscured by larger, overlying hypodermal cells. Approximately 75 microns from the anterior end is the first of two ventrally situated large cells of unknown function.

Baylis (1947) described the early appearance of these two large cells in Mermis nigrescens in a juvenile nine days old as "...a nucleus of a large cell (that) is visible in close contact with the ventral surface of the anterior stichocyte. This is probably the rudiment of two large cells which later appear in this position and are connected with the sheath of the oesophageal tube." These two cells apparently are differentiated earlier in M. subnigrescens since they are already seen, although with difficulty, in juveniles 5 days old, and without difficulty by the seventh day. The single nucleus described by Baylis (1947) was not apparent in M. subnigrescens. The second nucleus in the specimens of Baylis may have been obscured by the first of the closely adjacent rudimentary stichocytes which lie immediately posterior to these two cells in M. subnigrescens.

In the five-day-old juvenile, one binucleate stichocyte rudiment lies at the same level of the two cells of undetermined function noted above, and three stichocyte rudiments lie posterior to them. Collectively, the four rudiments measure approximately 100 microns in total length. Cell boundaries, if present, are difficult to distinguish. The anterior portion of the multicellular trophosome is apparent directly posterior to the last of the incipient stichocytes. The genital anlagen are seen as an opaque body in the anterior ventrolateral portion of the trophosome.

At six days, the juvenile is 400 microns long and measures 30 microns in maximum thickness in the area between the second and third stichocyte rudiment (Figure 41). The blunt anterior end is twelve microns in width; the posterior end, thirteen microns. Further development of the two anterior cells has occurred and these are now somewhat elongated and are considerably larger. The first of these two cells is now located 90 microns from the anterior. The second cell, of similar size, partially obscures the first of the stichocyte rudiments. These rudiments now have a combined length of 140 microns. The anterior extent of the trophosome appears 230 microns posteriad to the anterior end. Lying 25 microns posterior to the anterior extent of the trophosome is the genital anlage. Hypodermal chords are visible in the posterior half of the worm.

By the seventh day, the juvenile is approximately 790 microns long with a maximum width of 40 microns (Figure 42). The bluntly squared anterior end is 18 microns wide; the bluntly rounded posterior end is 15 microns wide. The first of the stichocyte rudiments is 145 microns from the anterior end and is almost obscured by the two large oblong cells ventral to it. Located 400 microns from the anterior end is the last stichocyte rudiment. Directly posterior to this cell and located 425 microns from the anterior end is the trophosome. The broadly rounded genital anlage is 490 microns from the anterior and is 35 microns in diameter. No increase in size of the stylet is apparent, and it remains approximately 12 microns long. A single pair of cellular masses lies approximately 75 microns from the anterior end, and apparently represents the first differentiation of the nerve mass. At this stage, the esophagus

is not distinguishable.

In the eight-day-old juvenile, little further differentiation has occurred (Figure 43). The average length is 1.3 mm, and approximately 100 microns from the anterior end are two elongated masses, the presumed nerve mass. Of the two large anterior cells, the first is 125 microns from the anterior end; the second, 15 microns posteriad to the first. The first stichocyte rudiment is 160 microns from the anterior. Evidence of separation is apparent in the four stichocyte rudiments, this separation occurring midway between the two clear nuclei of each rudiment. These four rudiments have a total combined length of 200 microns. The trophosome begins approximately 510 microns from the anterior end. Located 720 microns from the anterior is the genital anlage. The anterior end of the worm has become narrowed and now measures but 15 microns in diameter in contrast to a diameter of 50 microns approximately 0.10 mm from the anterior. Average maximum body width of 54 microns is found at the level of the third stichocyte rudiment.

By the ninth day, the juvenile is approximately 1.18 mm long but has not increased in thickness from the previous day, remaining approximately 56 microns in maximum thickness. In a juvenile 1.5 mm long (Figure 44), the first of the two large elongated cells is 100 microns from the anterior end and is 75 microns in length. An obvious change is the differentiation of the stichosome, whose cells now appear elongate and pyriform. The first of eight distinct stichocytes lies 140 microns from the anterior extremity; the eighth cell, 680 microns. Dorsal to the last stichocyte is the overlying anterior end of the trophosome. The genital

anlage, somewhat larger and more conspicuous than previously, is 875 microns from the anterior end. The esophagus is definitely present at this time and follows a tortuous course for approximately 200 microns before becoming obscured by the stichosome. Each of the stichocytes appears to be connected to its neighbor by a thin membrane. Whether or not the esophagus is formed within this membrane is difficult to demonstrate. Its position suggests that it may represent the rudiment of the developing esophagus, or perhaps it forms the sheath around the esophagus itself.

Juveniles 11 days old average 2.67 mm long, although several specimens exceeded 3.0 mm. A single 2.6 mm juvenile (Figure 45) had a maximum width of 75 microns. The average maximum width (in the area of the presumed nerve mass, 100 microns from the anterior) was 65 microns. Fifty microns from the rounded posterior end, the width was 48 microns. The first large cell is now 165 microns from the anterior and the eight stichocytes occupy an area 325 to 1150 microns from the anterior end. Beginning 860 microns from the anterior end, the trophosome now overlies the last three stichocytes. The esophagus can be followed for approximately 500 microns before it becomes obscured by the stichosome.

- More than a fourfold increase in average size occurs between the ninth and eleventh day juvenile. Baylis (1947) noted a similar increase in M. nigrescens between the ninth and thirteenth days, when juveniles quadrupled in length. He indicated that this sudden burst of rapid growth strongly suggested that a moult had occurred, but he observed no shedding of the integument. No shedding of integument was observed at this stage in this study.

After the twelfth day, juveniles appear to change little morphologically. Ventral and lateral chords of the hypodermis are obvious by this time, extending from the posterior end to about 150 microns from the anterior end. Hypodermal cells are clearly seen, but less so in the anterior half of the worm. Usually the esophagus can be followed as far posterior as the trophosome, but ultimately it becomes obscured by the trophosomal contents (Figure 46). The genital anlage is difficult to identify except in ventral or ventrolateral aspect because of the overlying dense trophosome.

By the thirteenth to fourteenth day, differentiated areas in the anterior end include the esophagus, nerve mass, and trophosome. The growth rate of the juvenile now becomes even more erratic than before. Eight juveniles 16 days old varied from a minimum length of 18.5 mm to a maximum of 36.6 mm, with a maximum width of from 130 microns to 184 microns.

Beyond the sixteenth day, details of internal structure are difficult to interpret since the trophosome now extends almost to the anterior end. No specimens were available between the sixteenth and twenty-seventh day, but at the twenty-eighth day and up to thirty-nine days, growth is very rapid and the parasitic juvenile increases in length from an average of 44.3 mm on the twenty-eighth day to an average of 93 mm on the thirty-ninth day.

From Table 6, it appears that some difference occurs in growth rate of M. subnigrescens and M. nigrescens, increases in length and width being greater in the former. There may be several explanations for this difference. There may be a real difference between the two species in

their growth rate. Baylis (1947) used the earwig Forficula auricularia as his experimental host, while in the present study Melanoplus femurrubrum, M. bivittatus, and occasionally M. differentialis were used. The type of host selected may influence to some extent the rate of growth. Baylis does not indicate the age of his hosts, nor does he indicate how many M. nigrescens eggs were fed to the hosts. The number of juveniles present in a host, the age of the host, and the availability of food may also influence the growth rate of the parasite.

A wide range in size of parasites of the same age may exist. Within a particular host, for example, the nine-day-old parasitic juveniles in a single specimen containing six juveniles had a minimum length of 790 microns and one juvenile had a length of 1050 microns representing a range of 260 microns. On the thirteenth day, the smallest parasite measured 4.83 mm and the longest 10.5 mm, a range of 5.67 mm within the same host. It is therefore difficult to determine precise age of the parasite using only measurements of length, which may be almost meaningless except to give an approximation of average growth rate.

Nevertheless, certain information may be deduced from these measurements. Baylis stated that a moult apparently occurs between the ninth and eleventh days. He bases this observation on the presence of "...an extremely delicate loose sheath of cuticle (which) can be seen at the extremities". He further stated that the sudden increase in growth suggested the occurrence of a moult. A similar rapid increase occurred in specimens in this study between the ninth and twelfth days and tends to support the theory that a moult has taken place. The lack of any indi-

cation of any shed integument, as stated by Baylis, and the fact that none was found in this study suggests that it perhaps is absorbed and that this absorbed integument may be used in the formation of a new body covering. That nematodes can absorb at least part of their old integument has been shown by Lee (1966b) and Bird and Rogers (1965). They demonstrated that at least a portion of the old integument is used to form the new. A similar process occurs in insects during their moults, although it should not be inferred here that the two processes are homologous.

The average lengths of parasites from various sizes of three species of grasshoppers used in experimental feedings are presented in Table 7.

Table 7. Comparison of lengths of M. subnigrescens in species of grasshoppers of varying size

Species	Parasite age (days)	Parasite length (av. in cm)	Grasshopper length (mm)	Average no. parasites
<u>Encoptolophis sordidus</u>	28	4.8	not determined	5.5
<u>E. sordidus</u>	35	2.4	1.5	15
<u>E. sordidus</u>	36	7.8	2.0	2.3
<u>E. sordidus</u>	37	7.1	1.7	4.5
<u>E. sordidus</u>	38	4.1	1.65	6
<u>E. sordidus</u>	39	3.25	1.7	13
<u>E. sordidus</u>	44	5.25	1.65	4.5
<u>Melanoplus differentialis</u>	39	13.1	4.45	7
<u>M. femurrubrum</u>	14	1.8	not determined	20
<u>M. femurrubrum</u>	28	4.6	"	7.6
<u>M. femurrubrum</u>	31	8.85	"	1.5
<u>M. femurrubrum</u>	34	8.7	3.6	4
<u>M. femurrubrum</u>	35	8.7	not determined	2
<u>M. femurrubrum</u>	37	7.2	2.8	3

In general, the above observations tend to indicate that length of the parasite is probably influenced by both size of the grasshopper and number of parasites within a host. Different grasshopper species probably have only minimal effect on size of the parasite, although those found in M. differentialis are larger than in corresponding age groups of M. femurrubrum, M. bivittatus and E. sordidus. This conclusion is supported by observations from dissections of M. differentialis found infected in nature. The largest parasites from M. differentialis are always much larger than the largest parasites from any of the above species. In general, the length of the parasite is directly proportional to the size of the host and probably inversely proportional to the number of parasites present.

Emergence

Christie (1937) reported that males of M. subnigrescens remained in the host from four to six weeks prior to emergence and attained a length of from 20 to 60 mm. Females usually remain in the host from eight to ten weeks and attain a length of 50 to 160 mm. However, in the present study, the maximum length of a female was approximately 135 mm. In other respects, results of the present study tend to agree with observations of Christie.

In my study, there seemed to be a tendency for male juveniles to predominate in heavy infections (over twenty juveniles per host). In infections of less than five or six individuals, usually all juveniles were female. Infections of from five to approximately fifteen juveniles usually produced mostly females, but one or more males may be present.

In two experimental hosts where emergence was observed, 12 and 16 juveniles respectively were obtained. It was noted that both males and females emerged at the same time. Emergence of juveniles from the host harboring 16 individuals occurred on the thirty-fifth day postexposure, and on the thirty-seventh day for the host containing 12 individuals. Generally, in infections where only males are present, the time of emergence is from two to six weeks earlier than in infections where only females occur. It was assumed that the larger individuals in these two cases were females. Although their postparasitic development was not followed, these female juveniles appeared healthy upon emergence, being as active as the newly emerged males. Similar observations on sex in mermithids have been reported by Christie (1929).

Integument

Juvenile integument, in experimental infections, remains extremely thin for the first 28 to 30 days (Figure 27). During this time, it is barely visible in cross section and averages approximately one micron in thickness up to the sixteenth day. At that time, it is not possible to differentiate clearly the integumental layers. By the twenty-eighth day, the integument has increased to a thickness of six to eight microns and between the thirty-third and thirty-sixth day, it suddenly increases to 12 to 15 microns in thickness. Shortly prior to the emergence of the juvenile from its host, the integument averages 27 to 32 microns and occasionally exceeds 35 microns in thickness. If the parasite absorbs its nutrients from the haemolymph of its host, this may account for the

rather thin integument during the early parasitic stage. The sudden increase in thickness of the integument may signal the end of nutrient absorption and may stimulate the parasite to escape from its host.

Layers of the integument present by the thirty-second to thirty-fifth day include the cortical layer, two fiber layers lying at approximately right angles to each other, and the thick, spongy matrix layer. The basal lamella is not distinguishable at this time. In cross sections of infected grasshoppers, it was noted that in some juveniles there appeared to be a loosening of the matrix from the underlying basal layer. Since these juveniles appeared to be about the stage of normal emergence (approximately 36-40 days post-exposure), this might be interpreted as the first indication of the forthcoming moult which generally takes place within two months after emergence. This seems unlikely however, because moulting in nematodes, according to Lee (1965b), does not take this long. This loosening of the integument may represent an artifact of fixation or sectioning. Other younger worms within the same host did not demonstrate it.

Hypodermis

Lying directly between the integument and body wall musculature and running the length of the worm are the thickened hypodermal chords (Figure 26). The number of rows of cells present in each chord varies in accordance with the body region and with the age of the worm. In juveniles 28 days old, there are only four distinct chords present. A narrow dorsal chord consists of a single row of cells. Two large prominent lateral

chords generally consisting of three rows of cells (but sometimes as many as five rows) may be present in the mid-body region. A ventral chord consists of two rows of cells. Hypodermal cells are very large with prominent nuclei. Hypodermal chords project into the pseudocoel and divide the body wall musculature into four muscle fields.

In the postparasitic juvenile, hypodermal chords increase in number. In the mid-body region, eight distinct chords can be seen, namely: one dorsal, two subdorsal, two lateral, two subventral and a single ventral chord. In the anterior end, the subdorsal chords are not seen and, anterior to the nerve mass, subventral chords are not visible. The four remaining chords, i.e., dorsal, two laterals, and ventral, are present to the anteriormost end of the worm. Posteriorly, the hypodermal chords are difficult to follow, but at least the two lateral chords and the ventral chord extend to the posterior end. Longitudinally, hypodermal cells of the postparasitic juvenile may measure 43 by 50 microns and their extensions project into the pseudocoel for 35 to 40 microns.

The hypodermis in juveniles of seven days is barely evident except in the posterior region where it is more clearly seen. The chords are not yet separate and distinct, but appear as a continuous peripheral band posteriorly. Baylis (1947) stated that hypodermal cells are well developed in M. nigrescens juveniles 11 to 13 days old. However, in M. subnigrescens, by the eleventh day, chords are well formed and extend from the posterior end to well past the mid-body region. Baylis inferred that the development of the trophosome parallels that of the hypodermal

chords and he supported the interpretation that the cells of the chords are involved in the collection or elaboration of food materials. Johnson (1955), indicated that hypodermal cells also give rise to the genital anlage in Hydromermis contorta. Since my cross sections of Mermis subnigrescens in the region of the genital anlage show it and a lateral chord to be continuous, Johnson's conclusion may apply here. Although the development of the genital anlage was not observed from its very beginning, numerous sections through the anlage show it always to be continuous with a lateral chord (Figures 6 and 7). Such evidence makes Johnson's viewpoint a very convincing one.

Body wall musculature

According to Chitwood and Chitwood (1937), body wall muscles of mermithids are polymyarian and coelomyarian. Eight muscle fields occur in the mid-body region of juveniles 32 days old, and this number apparently remains constant thereafter. Anteriorly (in the anterior one-eighth of the body), the number of muscle fields is reduced to six due to the absence of subdorsal hypodermal chords.

In juveniles of seven days, no differentiation of recognizable muscle is apparent, but muscle rudiments are present in the form of vase-shaped cells (Figure 5). By the sixteenth day, body wall musculature is beginning to differentiate into muscle fibers widely spaced within a muscle field.

In the 28-day-old juvenile, muscles are clearly differentiated. In cross section, each muscle field projects ten to fifteen microns into the pseudocoel. Muscle fibers are grouped more closely, but there are

still spaces between adjacent groups of muscle fibers. Later, in post-parasitic juveniles and adults, the muscle fibers become packed within a muscle field and no spaces are evident.

Trophosome

In young juveniles (seven to eleven days), the trophosome appears as a cylinder of small undifferentiated granules extending from the posterior stichocyte rudiment to the posterior end of the juvenile (Figure 42). Scattered throughout are numerous oval cells. The trophosomal wall is not clearly distinguished. A parenchymatous-like matrix or network is evident throughout the trophosome. By the ninth day, the anterior end of the trophosome begins to overlies the posterior stichocyte rudiment.

After the sixteenth day, the trophosome, especially the posterior region, appears to become vacuolated. Cross sections of such juveniles confirm that numerous vacuolated areas exist. By this time, a few trophosomal spheres have been formed, perhaps from the granules noted earlier. Many of these spheres contain crystal-like aggregations. The vacuolated matrix appears to surround the spheres. Extensions of this network originate from the now visible trophosomal wall. By the twentieth day, the anterior end of the trophosome has completely overlapped dorsally the posterior six stichocytes.

In juveniles of 28 days, the trophosome extends almost to the anterior extremity terminating just short of the nerve mass.

Baylis (1947) stated that in M. nigrescens, the trophosomal spheres do not appear until after the thirty-first day. He does not state if he

examined cross sections of younger juveniles, but apparently observed these spheres only from in toto specimens. It seems strange that Mermis subnigrescens and M. nigrescens should be so similar in development in most instances, but apparently so different in the time of formation of trophosomal spheres.

Just prior to emergence of the parasite from the host, most of the vacuolated spaces are filled with trophosomal spheres, which now occupy the entire trophosome. The post-parasitic juvenile similarly shows little evidence of vacuolated areas, although a few persist. The trophosomal wall apparently consists of a syncytial tissue with many nuclei on its periphery.

The esophageal apparatus

That portion of the digestive tract commonly referred to as the esophagus (Figure 53) in mermithids has perplexed zoologists for many years. That this apparatus differs from what is normally seen in nematodes is well known. Dujardin (1842) described it, in M. nigrescens, as being continuous with the anterior portion of the intestine (trophosome). Meissner (1856 Tafel I, Figure 7) illustrated the esophageal apparatus as having "Magenhöhlen" (presumably lateral outpocketings as indicated in his illustration) along its course and projecting into the trophosome. He was the first to observe that the esophagus ended blindly in the posterior half of the worm. Von Linstow (1892) also observed that the esophagus ("Chitinrohr") ended blindly. He also described cells (known currently as stichocytes) containing what he believed to be glandular masses in which is embedded a body (esophageal sheath ?) containing the "Chitinrohr".

Additional information on the esophagus of other mermithids was presented in some detail by Müller (1931) and by Christie (1936).

Steiner (1929) described at least three types of cells around the esophageal tube of mermithids. He concluded that these cells may be excretory or may have to do with the "Abbau" of the trophosome and the change of its stored material into sexual products. He doubted that the apparatus is functionless, but indicated that it could not serve as an inlet for food because of a lack of musculature around the tube. Chitwood (1935) claimed that each stichocyte had an opening into the esophageal tube, and was, in fact, an esophageal gland. Christie (1936) gave a detailed description of the esophageal apparatus as found in Agamermis decaudata, but the presence of sixteen stichocytes and other differences in the apparatus makes this an abnormal situation among mermithids.

There was no evidence in this study of any openings in the esophageal tube, although Chitwood and Chitwood (1937) illustrate it as possessing numerous lateral openings. According to Baylis (1947), there was little doubt that the entire apparatus in M. nigrescens was of a glandular nature because secretions were voided through the mouth. He further suggested that the secretion had a proteolytic action which assisted the juvenile in emergence from its host. In the present study, it was noted that the parasite always emerged from the host anterior end first. This might indicate that a proteolytic substance may be secreted from the anterior end.

It must be concluded that the function, if any, of the esophageal apparatus and stichocytes remains enigmatic.

Genital anlage

The genital anlage, apparently derived from the lateral hypodermal chord, first appears as a solid mass of tissue lacking any internal differentiation (Figure 6). In cross sections of juveniles 28 days old, the genital anlage appears granular (Figure 7). Morphological differentiation of sex is not apparent until just before emergence of the juvenile from its host when a spicule anlage is present in males. Following the post-parasitic moult, the female genital region becomes sufficiently differentiated to distinguish the vulva, vagina, and the two uteri. The vulva, located midway on the body, is slightly elevated and quite prominent. The vagina, or egg-ejector according to Cobb (1926a), extends into the body for approximately two-thirds of its diameter and then bends anteriorly an equal distance before it receives the two less muscular uteri (Figure 8). A vaginal sphincter muscle may be seen at the junction of the uteri with the vagina and apparently regulates the rate of egg deposition. The sphincter muscle can be seen to contract rhythmically during egg laying.

Spicules of the male become evident after the first postparasitic moult. The testis and sperm duct apparently complete their development at this time.

Effects of the Parasite on the Host

Early infections by M. subnigrescens juveniles appear to have little or no visible effect on the behavior or mobility of the host grasshopper. Even in experimental infections where more than 70 eggs were fed to nymphs,

visible indications were observed even though, when later dissected, from 35 to 40 parasites could be found. No attempt was made to determine if grasshoppers could ingest a lethal dose of mermithid eggs, but the lethal dose must be substantially more than 70 eggs to cause death within a reasonable length of time. Unfortunately, in the experimental infections in which more than 70 eggs were given at one time, the test was concluded by the end of the 28th day. Several grasshoppers died during the course of this heavy infection, but it could not be determined if mermithids were the cause of death. Most of the deaths of hosts occurred during the latter half of the experiment. Grasshoppers caught in nature, and harboring over 65 juveniles, showed no noticeable effects externally due to the parasites.

Parasitic juveniles apparently do not overwhelmingly prefer any particular portion of the haemocoel or other body structures. Johnson (1955) reported that Hydromermis contorta prefers the posterior portion of the chironomid body until late in the infection when the disappearance of the fat bodies at the anterior end of the host provides the parasite with added space in that region. However, M. subnigrescens seems to migrate throughout the body of its host, for it is not unusual to find young juveniles looped around the tentorial arms, among the mandibular adductor muscles, in the metathoracic leg, and penetrating the dorsal and ventral diaphragms of the insect host.

Since parasitic juveniles seem to be found in most internal structures except the alimentary canal and oviducts of its host, what effect, if any, is there on the host?

Generally, there is no macroscopic external indication of an infection. Johnson (1955) reported that chironomids parasitized by Hydromermis contorta have a pale body instead of the usually bright red body. Christie (1936, 1937) observed no color change in parasitized grasshoppers, and infected specimens in this study supports that observation. Wheeler (1907) noted numerous morphological changes in ants parasitized by mermithids, but apparently none of these changes are present in grasshoppers infected with Mermis subnigrescens. The posterior ends of a number of normal and infected grasshoppers were mounted and observed microscopically. No apparent differences were noted.

The mandibular adductor muscle of grasshoppers consists of several bundles of fibers. When a parasitic juvenile is present in the bundles, the bundles appear to be smaller and more compressed than normal (Figure 9).

When the worm is found in the region of the brain, the protocerebrum and deutocerebrum appear to be slightly atrophied (Figures 10 to 12).

A section through the salivary glands (Figures 13 to 15) shows these structures to be markedly reduced and generally more darkly stained in parasitized individuals. Johnson (1955) reported similar observations in chironomids parasitized by Hydromermis contorta.

In areas where the parasitic juvenile has penetrated the dorsal musculature of the body wall of the abdominal region of the grasshopper, there appears to be compression (Figure 16) and atrophy of the muscle bundles.

When a parasite is lying apposed to the gut wall, that immediate area

of the gut seems to be thinner than is a comparable area when no parasite is present (Figure 17).

The destruction of much of the fat body is evident in almost all examples of infection (Figures 18 and 19). Johnson (1955) found a similar result in his work.

Many examples of mechanical distortion of the gut and lateral oviducts (Figures 20 and 21) and gut were encountered. This distortion consisted of compression and partial closure of the lumen of these structures due to crowding of the parasite against the outside wall of these structures.

Christie (1936) stated in every case where he had examined an adult female Melanoplus femurrubrum parasitized with Agamermis decaudata, that the ovaries were reduced in size and in many cases were vestigial. He also stated that similar results were found in grasshoppers parasitized by M. subnigrescens (Christie, 1937).

Many parasitized female grasshoppers were examined histologically as well as in toto in this study. Mermis subnigrescens were usually found in the area of the ovaries and were often found threading their way between individual ovarioles. This usually caused the latter to be reduced somewhat in size. In no instance, however, were they so reduced that they could be called vestigial, nor were they ever found in the condition illustrated by Christie (1936, Figure 13). It was noted that in several cases, there was evidence that the eggs in the ovarioles appeared to be misshaped in the vicinity of the parasite. In extreme cases, these eggs may not have been viable.

The evidence found in this study seems to indicate that there may be a reduction in the number of eggs present in parasitized female grass-

hoppers when compared to normal grasshoppers of the same age and species. This reduction in egg number may be real or the parasite may retard the development of host eggs so that they may not mature until a later period subsequent to resumption of normal egg production.

Parasitized males (Figure 22) showed very little difference in the development of the testes when compared to normal males. Christie (1936) is of the opinion that most, if not all, parasitized male grasshoppers are capable of producing spermatozoa and the evidence from this study tends to support this opinion.

Several specimens of grasshoppers carried a mermithid which had penetrated the neurilemma or nerve sheath (Figure 23). In these cases, the parasite was apposed to the nerve cord but there appeared to be no damage to the nerve cord itself. Reports that insects parasitized by mermithids lose their irritability or become immobile (Johnson, 1955; Christie, 1936) may be due to the parasite's coming in contact with the nerve fibers by penetrating the neurilemma.

Reports of damage to the reproductive organs are numerous (Christie, 1936, 1937; Smith, 1965; Riley, 1878) and these reports usually infer that this reduction is due to mechanical damage done by the parasite. Another theory is that the parasite may absorb such a quantity of nutrients from the haemolymph that the remaining nutrients in the haemolymph are insufficient for the normal production of eggs (Baylis, 1944, 1947). Both of these theories are probably correct, but yet a third factor may be involved. Exudates from the parasite may be toxic to normal ovarian development. An experiment was conducted to test this hypothesis.

A population of Melanoplus femurrubrum and Melanoplus bivittatus was divided into five experimental groups, each containing ten males and ten females. Group I served as normal controls, Group II was given a sham injection with a hypodermic needle only, Group III was given an injection of modified Ringer's insect saline, Group IV was given an injection of exudate in modified Ringer's insect saline, and Group V was given an injection of worm extract in modified Ringer's saline. Not all insects survived, but in no instance did less than seven females survive. Procedures employed in this experiment have been given in the section on materials and methods. Results are presented in Table 8.

Table 8. Effects of parasite exudate and extract on grasshopper egg production

Group	No. female hosts (<u>Melanoplus</u> spp.)	Total no. eggs laid	No. eggs per host
I	8	797	99.6
II	7	605	86.4
III	8	895	112.0
IV	8	491	61.4
V	7	358	51.1

Both Melanoplus femurrubrum and M. bivittatus lay approximately the same number of eggs. Riley (1878) reported that M. femurrubrum lays from 96 to 100 eggs in a season. Similar results have been reported by Hewitt and Barr (1967) and Severin and Gilbertson (1931) for both of these species.

Grasshoppers in each of the above five groups were of similar age. There appears to be a significant reduction in the number of eggs found in Groups IV and V due to the presence of some product or products from the worm. Unfortunately, it was not possible to make a chemical determination of the worm exudate at this time, but this should be done. A much larger population of grasshoppers should also be used to accurately determine the effects of the exudate and extract on the host.

Three factors, namely: mechanical damage, depletion of nutrients and toxic products from the parasite may all contribute to the reduction in the number of eggs by female grasshoppers parasitized by mermithids.

Numerous workers, including Christie (1936), Johnson (1955), and Phelps and DeFoliart (1964), have reported that the host dies within several hours after the emergence of the parasite. The present study verified these reports.

Often an infected grasshopper may die before the emergence of its contained parasite(s). Numerous dead grasshoppers still harboring parasites that were judged to be almost at the stage of emergence were found in various stages of post-mortem deterioration. Why these older aged juveniles had not escaped from the host when it died is difficult to understand. Perhaps the worm produces some type of proteolytic substance just before emergence under normal conditions which enables it to penetrate through the body wall of the host. If the grasshopper dies before this occurs, the mermithid may be unable to free itself from the dead grasshopper body. However, in such instances, the worm seems to have a protective mechanism. If an infected grasshopper has been dead for

several days and is dry and brittle, the parasite within is also hard, dry and reduced in size by apparent dehydration. Bodies of such seemingly dead parasites, when placed in water, will quickly absorb water and the individuals will gradually revive again and regain their normal size. Within an hour they may begin normal movements. Such parasites continue to live as normal postparasitic juveniles, but whether or not they are capable of complete development to adulthood was not determined. This striking phenomenon of apparent rejuvenation was repeatedly observed and deserves further investigation.

These studies indicate that in nature, when an infected grasshopper dies before the mermithid emerges, the latter may undergo dehydration and remain in this state in the decomposing host until moisture revives the parasite. Such an individual then frees itself from its host, enters the soil, and probably continues the normal life cycle.

Adults

Adults of Mermis subnigrescens have been described in detail by Cobb (1926a). Cross sections of M. subnigrescens show no significant morphological differences from published illustrations of cross sections of M. nigrescens by Dujardin (1842), Meissner (1856), von Linstow (1889, 1892, 1899), Hagmeier (1912), and Müller (1931). Representative cross sections of adult M. subnigrescens derived from experimental infections are shown in Figures 24 to 26.

Taxonomy of M. subnigrescens and M. nigrescens

The validity of the two mermithid species, namely M. nigrescens and M. subnigrescens was questioned by Cobb (1926a), when he established M. subnigrescens as distinct from M. nigrescens. Following the description of his newly established species, he stated "...only further research can determine whether the names nigrescens (and) subnigrescens should denominate specific, varietal, or conceivably, only racial differences."

Baylis (1944) compared the two species, but few papers have given additional information about them. The following data corroborate the viewpoint of Cobb and include further evidence that they are distinct and separate species.

In all reports of infection in which M. subnigrescens has been accurately identified, the host has always been a member of the Order Orthoptera. M. nigrescens has been reported from this order as well as from a number of other insect orders (Appendix).

Baylis inferred that M. nigrescens does not come to the surface to lay eggs more than once in a season. M. subnigrescens, as demonstrated by Christie (1937) and verified in the present study, will deposit its eggs several times during a season in nature as well as under laboratory conditions.

Baylis reported that postparasitic females of M. nigrescens from Orthoptera varied in length from 106-172 mm, while similar females of M. subnigrescens were reported by Cobb to measure 67-163 mm in length. In the present study, 15 female postparasitic juveniles of M. subnigrescens varied from 85-135 mm and averaged 98 mm. Seemingly, the postparasitic

females of M. nigrescens are somewhat longer than those of M. subnigrescens.

The vulva of M. nigrescens, as described by Dujardin (1842), is located far anteriorly in the female. Cobb (1926a), in his study of M. subnigrescens, described the vulva as situated near the middle of the body. Measurements of 15 postparasitic females in the present study indicate that in the average 98 mm worm, the vulva is located 50.3 mm from the anterior end. These measurements, then, tend to support Cobb.

Morphological differences between the postparasitic stages of these two species of Mermis, other than the position of the vulva and total length, seem to be few and of little significance.

Evidence from the present study seems to indicate that the growth rate in the parasitic juvenile of M. subnigrescens is more rapid than that of M. nigrescens juveniles (Table 6), especially during the first two weeks after emergence from the egg. Internal organization, including the formation of stichocyte rudiments and hypodermal chords, appears to occur earlier, often three days earlier, in M. subnigrescens than in M. nigrescens.

From the above comparisons, especially with reference to the position of the vulva, host preference, growth rate in the early parasitic stages, and earlier differentiation of the stichocytes, it appears likely that Cobb was correct in his opinion that M. subnigrescens is a valid species and should be separated from M. nigrescens.

However, conclusive evidence to substantiate the separation of the two species would be provided by the feeding of eggs of both species to the same

species of host and by a comparison of the morphology of the parasitic juveniles, the postparasitic juveniles, and mature adults. Such evidence, unfortunately, is not yet at hand.

HISTOCHEMISTRY

Postparasitic Juveniles

A series of histochemical studies was undertaken to determine the chemical composition of certain structures of postparasitic juveniles of Mermis subnigrescens. Organs and tissues included the integumental layer, hypodermal layer and chords, body wall musculature, trophosomal wall, trophosomal spheres (globules of Chitwood and Jacobs, 1938), supporting tissue surrounding the spheres, and, in addition, the esophagus, esophageal cells, ovary and ovarian epithelium.

Worms used were juveniles from experimentally infected grasshoppers fed M. subnigrescens eggs which had emerged from their hosts approximately four months prior to use in these studies. These juveniles were kept in moist soil and were maintained at a temperature of 15-18° C. Standard histochemical techniques, described in detail by Pearse (1960) and by Lillie (1965), were used throughout. Sections of paraffin embedded tissue were cut at a thickness of from five to eight microns on a rotary microtome. Other sections were prepared from fresh or neutral formol fixed tissues by use of an International CT I cryostat, and were cut at five to fifteen microns at -23° C. All histochemical tests below are taken from Pearse (1960) except as otherwise noted. The nomenclature of Chitwood and Chitwood (1937) was used for the layers of the layers of the integument.

The most useful test for proteins was the mercury-brom-phenol-blue (MBPB) method as described by Bonhag (1955). Alkaline fast green reaction

was used for determining the presence of basic proteins. Millon's reagent was used to reveal proteins containing tyrosine; the Sakaguchi reaction (Figure 27) for arginine; dimethylaminobenzaldehyde-nitrite (DMAB-nitrite) and the rosindole reaction of Glenner (Pearse, 1960) for indicating evidence of tryptophan. Structural proteins in the integument were detected by use of van Gieson's stain for collagen and by Weigert's as well as Verhoeff's stain for elastin. Dihydroxy-dinaphthyl-disulfide (DDD) was used to demonstrate sulfhydryl (-SH) groups and the ferric-ferricyanide method of Chevrement and Frederic (Pearse, 1960) was also used to demonstrate sulfhydryl groups in addition to determining the degree of keratinization. The performic acid-alcian blue (PAAB) reaction was used to reveal the presence of disulfide (-S-S-) groups. Tyrosine was demonstrated by the Morel-Sisley diazotization method. The presence of the mucoprotein amyloid was tested by using the dahlia and the modified (Pearse) methyl violet methods.

Lipids were demonstrated by the use of neutral formol fixed cryostat sections in the following tests: Oil Red O in acetone-alcohol, Oil Red O in propylene glycol, Clark's Scharlach R, osmium tetroxide, Sudan Black B in propylene glycol, the Nile Blue reaction for neutral fats and fatty acids, and the Lorrain, Smith-Dietrich method for lipoids. All solutions were prepared according to the Gridley Manual of Histologic and Special Staining Techniques (1960). Control sections were prepared by pyridine and alcohol-ether extraction methods. Paraffin sections were used to reveal the presence of bound lipids by the acetone-Sudan Black B method and the burnt Sudan Black B method. The Nile Blue method of Cain (Pearse,

1960) was used to demonstrate neutral and acidic lipids, and the pseudo-plasmal reaction was used for phospholipids. 'Tween 60 and 'Tween 80 methods were used as tests for lipase.

For the demonstration of carbohydrate containing substances, the periodic acid-Schiff (PAS) reaction was employed on paraffin sections fixed in neutral formol. Controls for this technique included blocking by acetylation, unblocking by deacetylation, and exposing sections to PAS reagent without prior oxidation in periodic acid. Acetylation was carried out in the acetic anhydride-pyridine solution (Pearse, 1960) for at least four hours. Shorter periods did not result in complete acetylation of the tissue. Deacetylation was done in a 0.1% KOH in 70% alcohol solution for thirty minutes as recommended by Lillie (1965). However, periods of 15 minutes seemed to deacetylate five-micron-thick sections, and periods of more than 40 minutes in the solution resulted in the loss of many sections from the slide. Glycogen was demonstrated by Best's carmine stain and by the PAS reaction on sections treated with saliva, on those treated with 0.1% diastase in pH 6 phosphate buffer for 25-30 minutes at 37° C., and on sections treated with α -amylase digestion in a 0.5% concentration in four millimolar acetate buffer at pH 5.7 for 40 minutes at 37° C. The carbohydrate moiety was further characterized with alcian blue staining and metachromasia was shown by the thionin and toluidine blue methods.

Nucleic acids were detected by the pyronin-methyl green method on material fixed in neutral formol or in Carnoy's fixative and sectioned in paraffin. Only the integument was used in this study.

All tests were first run on vertebrate and/or invertebrate tissues

known to give positive reactions to specific substances.

Results of these histochemical reactions are given in Table 9 (Proteins), Table 10 (Lipids), and Table 11 (Carbohydrates).

The integument stained only slightly with the MBPB reaction, and moderately with the alkaline fast green stain for basic proteins. Much of the protein appears to be collagenous, as shown by the results of staining with van Gieson's stain. Excellent evidence has been presented by other workers for the occurrence of collagen in the integument of nematodes (Picken et al., 1947; Faure-Frémiet and Garrault, 1944; Flury, 1912; Kan and Davey, 1968; and von Brand, 1966). In this study, elastin could not be revealed in the integument either by the Weigert or Verhoeff stains. Amyloid also failed to be demonstrated in the integument.

Arginine occurs in all layers of the integument and is probably responsible for the basic protein staining. A high ratio of arginine is usually necessary for keratinized proteins (Savel, 1955). All layers of the integument except the matrix gave a moderate reaction for -SH and -S-S- groups. Control sections for sulfhydryl groups were treated by the saturated aqueous mercuric chloride methods of Yao as found in Pearse (1960). Sulfur containing amino acids may be due to the presence of keratinized proteins, as shown by Kan and Davey (1968) and by von Brand (1966). The presence of arginine and sulfur-containing amino acids was observed in the cortical and fiber layers and to a lesser degree in the matrix (Figure 28). Kan and Davey (1968), using identical histochemical methods employed in this study, found similar results for Phocanema decipiens. However, Lillie (1965) comments that methods used to distin-

Table 9. Proteins^a; postparasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
MBPB	+	+	+	+	+	++	+++	+++	-
Basic protein	++	+	+	++	+	++	++	++	++
DMAB-nitrite	-	-	-	-	-	-	+	+	-
Millon's	-	-	-	-	-	-	+++	++	-
DDD	++	+	+	++	++	++	++	-	?
PAAB	+	+	+	+	+	+	+	+	-
Sakaguchi arginine	++	++	+	+	++	+++	++	++	0
Tryptophan	-	-	-	-	-	++	++	++	0
Tyrosine	-	-	-	-	-	-	++	+	-
Van Gieson	+	++	++	-	-	-	-	-	-
Weigert	-	-	-	-	-	-	-	-	-
Verhoeff	-	-	-	-	-	-	-	-	-
Amyloid	-	-	-	-	-	-	-	-	-
RNA	-	-	-	++	+++	++	+++	++	++

^a+++ = strong reaction; ++ = moderate reaction; + = weak reaction; _ = faint reaction; - = negative reaction; ? = conflicting or uncertain reaction; 0 = tissue not observed.

Table 10. Lipids^a; postparasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
Pyridine control-oil	-	-	-	-	-	-	-	-	-
Red O	-	-	-	-	-	-	-	-	-
Oil Red O	-	-	-	-	-	+	-	+++	++
Alcohol-acetone									
Scharlach R control	-	-	-	-	-	-	-	-	-
Scharlach R	-	-	-	-	-	+	-	+++	+
Nile Blue	-	-	-	-	-	-	-	+++	+
Osmium	?	?	-	-	-	+	-	+++	+
Lipoids	-	-	-	-	-	-	-	+	-
Acid lipids	-	-	-	-	-	-	++	+++	-
Bound lipids	-	-	-	+	+	+	+	+	-
' Tween 60 and 80	-	-	-	-	-	+	-	+	-

^a+++ = strong reaction; ++ = moderate reaction; + = weak reaction; + = faint reaction; - = negative reaction; ? = conflicting or uncertain reaction.

Table 11. Carbohydrates^a; postparasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
PAS	++	+	+	+++	++	+++	-	-	++
PAS without periodic acid	-	-	-	-	-	-	-	-	-
PAS after acetylation	-	-	+	+	+	-	-	-	-
PAS after de-acetylation	++	+	+	+++	++	++	-	-	++
PAS after saliva	-	-	+	+	+	-	-	-	+
PAS after diastase	+	-	-	+	+	-	-	-	-
Alcian Blue	+	+	-	-	-	-	-	-	-
Acid mucopoly-saccharides	+	+	+	-	-	-	-	-	-
Toluidine blue	β metachromasia	β	β	0	0	0	0	0	0
Methylene blue extinction	Above pH 5	Above pH 5	Above pH 5	0	0	0	0	0	0
Best's carmine	+	+	+	++	++	+	-	-	-
Best's carmine with diastase	+	-	-	+	+	+	-	-	-

^a+++ = strong reaction; ++ = moderate reaction; + = weak reaction; + = faint reaction; - = negative reaction; ? = conflicting or uncertain reaction; 0 = tissue not observed.

guish protein-bound disulfide (-S-S-) and sulfhydryl (-SH) groups are to be accepted with great caution.

In the present study, the rosindole, indole, and tryptophan tests were negative in all integumental layers. Von Brand (1966) reported similar results for Ascaris lumbricoides, the only nematode listed by him.

The muscle, hypodermis, ovary, large and small trophosomal spheres, all gave positive reactions to the MBPB reaction and the alkaline fast green stain for basic proteins. The DMAB-nitrite test for tryptophan was positive only in the trophosomal spheres, and to a lesser extent, in the esophageal cells. A strong tyrosine reaction was shown by the trophosomal spheres. Most of the spheres stained moderately for arginine but muscle stained intensely and the ovary only lightly. The rosindole and indole reaction was lightly positive in the ovary. The -SH group was found in the hypodermal layer and chords, in muscle and only faintly in the larger trophosomal spheres.

In general, all layers of the integument stained with the PAS reaction, indicating the presence of carbohydrates (Figures 29-30). Small amounts of glycogen were present as indicated by a diminished reaction following digestion by saliva, diastase, and α -amylase. Alcian blue, generally considered specific for acid mucopolysaccharides, gave only a slight reaction in the cortical and two fiber layers. That carbohydrates are definitely present in the integument was shown by the reduction in staining after acetylation (Figures 31-32), and the restoration of staining after the acetylated material underwent deacetylation. The hypodermal layer chords, and muscle all showed a strong positive PAS reaction. The strong

positive PAS reaction was diminished by acetylation, diastase, and α -amylase digestion tests indicating the presence of glycogen in the hypodermis, body wall musculature, and to a lesser degree in the integument. Similar results were also found in the supporting tissue surrounding the spheres of the trophosome and to a lesser extent in the trophosomal wall and the ovary. The epithelium of the ovary revealed a strong positive PAS reaction. Similar results have been reported by von Brand (1966) in other species of nematodes. None of the trophosomal spheres reacted with any of the carbohydrate test reagents and therefore must be regarded as containing no demonstratable carbohydrates. The staining reactions with Best's carmine stain tended to support the results obtained with the PAS reaction.

In all tests for lipids, their presence in specific structures was difficult to demonstrate, principally because of a tendency for the colors to diffuse (Figure 35). However, by using teased sections, together with normal sections, certain structures could be demonstrated as containing lipids. Small trophosomal spheres were heavily colored in all tests for lipids. Large spheres were all negative except for demonstration of bound lipids where a slight reaction occurred, and a test for acid lipids where the large spheres reacted moderately. The supporting material surrounding the spheres gave a slight positive reaction. All other substances including the integument, gave a negative reaction to a variety of lipid tests (Table 10). Other workers testing for lipids have also failed to reveal their presence in the integument of nematodes (Lee, 1960). A slight reaction to bound lipids was given by the hypodermal layer and

chords and by the muscle layer in this study. One should accept the test for bound lipids with some caution. There is some evidence that acid lipids may occur in all trophosomal spheres. Nevertheless, any characterization of lipids by use of histochemical tests must be interpreted with caution. These tests do, however, provide a basis for more definitive work utilizing gas chromatographic techniques.

Lipase, as demonstrated with the 'Tween 60 and 'Tween 80 methods, showed some activity in all of the spheres and their supporting tissues.

No RNA was detected in any of the layers of the integument. Although RNA has been demonstrated in the integument of a number of nematodes (Anyia, 1966; Lee, 1961, 1962, 1965a); Kan and Davey (1968) failed to demonstrate it in the integument of the last juvenile and in the adult stage of Phocanema decipiens. The existence of RNA in areas other than the integument of M. subnigrescens was easily demonstrated, particularly in the trophosomal spheres.

Parasitic Juveniles

A series of histochemical studies was also carried out to determine the chemical composition of certain structures of the parasitic juvenile of M. subnigrescens. The results of these studies were compared with those from similar studies on postparasitic juveniles.

Worms for these studies were parasitic juveniles dissected from laboratory reared grasshoppers fed M. subnigrescens eggs 34 days previously. Standard histochemical techniques were identical to those used for the postparasitic stage. However, several tests used for the post-

parasitic juveniles were not included in studies on parasitic juveniles (see Tables 12-14). Interpretation of results of histochemical tests used for parasitic juveniles are based on the same criteria used in studies on postparasitic juveniles and are presented in Table 12 (proteins), Table 13 (lipids), and Table 14 (carbohydrates).

Discussion

In general, results of histochemical tests for proteins of parasitic juveniles differed only in relative amounts present found in postparasitic juveniles. Interestingly, in the parasitic juvenile stage, amino acids were relatively less abundant in the larger trophosomal spheres, and tests for general protein indicated relatively less protein in these spheres. Also, smaller trophosomal spheres gave only slight indication for tyrosine and general protein. There was a negative reaction for other amino acids and for protein tests. In the postparasitic juvenile larger and smaller spheres gave a strong positive reaction for general proteins and moderate to weak reactions for amino acids tested.

Comparison of tests for lipids in both juvenile stages revealed essentially the same lipid composition of the small spheres and the tissue supporting these spheres (Figure 36). Results of the Nile Blue test showed that in parasitic juveniles, there was relatively greater abundance of fatty acids than of neutral lipids, while in postparasitic juveniles, it was estimated that there was approximately equal amounts of both lipid forms (Figure 37).

Tests for carbohydrates indicated no substantial differences between parasitic juvenile and postparasitic juvenile stages. It is estimated

Table 12. Proteins^a; parasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
MBPB	0	+	+	+	++	++	+	+	+
DMAB-nitrite	0	-	-	-	-	-	+	+	-
Millon's	-	-	-	-	-	-	+	?	-
DDD	-	-	-	-	-	-	+	-	-
PAAB	?	?	-	-	-	-	-	-	-
Sakaguchi- arginine	-	-	-	+	+	+	++	+	0
Tyrosine	0	++	+	+	+	+	+	0	+
Tryptophan	0	-	-	-	-	+	+	0	0
Van Gieson	0	++	+	-	-	-	-	-	-
Weigert	0	-	-	-	-	-	-	-	-
Verhoeff	0	-	-	-	-	-	-	-	-

^a+++ = strong reaction; ++ = moderate reaction; + = weak reaction; _ = faint reaction; - = negative reaction; ? = conflicting or uncertain reaction; 0 = tissue not observed.

Table 13. Lipids^a; parasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
Pyridine- Scharlach R control	-	-	-	-	-	-	-	-	-
Scharlach R	-	-	-	-	-	+	-	+++	-
Oil red O	-	-	-	-	-	+	-	+++	-
Nile blue	-	-	-	-	-	-	-	+++	-

^a+++ = strong reaction; + = weak reaction; + = faint reaction; - = negative reaction.

Table 14. Carbohydrates^a; parasitic juveniles

Test	Integument			Hypodermal		Body wall musculature	Trophosomal spheres		Trophosomal wall
	Cortical	Fiber	Matrix	Layer	Chord		Large	Small	
PAS	+	+++	+	+	++	+	-	-	++
PAS without periodic acid	-	-	-	-	-	-	-	-	-
PAS after acetylation	-	-	-	-	-	-	-	-	-
PAS after de- acetylation	<u>+</u>	++	+	<u>+</u>	++	+	-	-	++
PAS after diastase	<u>+</u>	+	-	-	<u>+</u>	<u>+</u>	-	-	+
Best's carmine	0	+	+	+	++	+	-	-	+
Best's carmine after diastase	0	-	-	-	<u>+</u>	<u>+</u>	-	-	-
Methylene blue extinction	Above pH 4.5	Above pH 4.5	Above pH 4.5	0	0	0	0	0	0
Acid mucopoly- saccharides	-	-	<u>+</u>	-	-	-	-	-	<u>+</u>

^a+++ = strong reaction; ++ = moderate reaction; + = weak reaction; + = faint reaction; - = negative reaction; 0 = tissue not observed.

that there is a relatively greater amount of glycogen present in the post-parasitic juvenile stage than in the parasitic juvenile stage (Figures 38-40).

From the foregoing histochemical tests, it appears that the integument of Mermis subnigrescens is composed of a variety of substances. Collagen seems to be present in all layers of the integument, especially in the matrix layer, as shown by the results of staining with van Gieson's stain. The cortical layer contains a keratin-like protein, but probably is not true of keratin. True keratin, a protein with molecules cross-linked by cystine bridges, probably occurs only in vertebrates (von Brand, 1966). Keratin-like proteins have been described from the external cortical layer of Ascaris lumbricoides integument and from several other nematodes (Chitwood and Chitwood, 1937). The presence of arginine and sulfur-containing amino acids was confirmed in my studies and gave added evidence of a keratin-like protein in the cortical layer.

Carbohydrates are definitely present in the integument. However, the identification of these carbohydrates was not entirely satisfactory. A small amount of glycogen is probably present as well as mucopolysaccharides. The presence of mucopolysaccharides, as shown by alcian blue staining, appears to be only in the cortical and fiber layers (Figure 28). Similar studies by Marzullo, Squodrini, and Taparelli (1957), as reported in von Brand (1966) on Ancylostoma duodenale and on Enterorobius vermicularis reveal acid mucopolysaccharides in the integument of these two parasites.

The capacity to bind methylene blue below pH 4, according to Pearse

(1960), almost certainly indicates the presence of sulfate groups, a characteristic of acid mucopolysaccharides. All three integumental layers were observed to bind methylene blue below pH 4.

Lipids do not appear to be an important constituent of the integument, for only with the use of osmium tetroxide could a weak reaction for them be observed. With osmium tetroxide, it appeared that either the cortical and/or part of the fiber layer stained, but this was difficult to distinguish. The use of other lipid stains failed to demonstrate lipids in the integument. Kan and Davey (1968), in their studies of Phocanema, and Lee (1960), in his studies of Thelastoma bulhoesi and Nippostrongylus brasiliensis, reported the absence of lipids in the integument of these nematode parasites.

The body wall muscles and hypodermal chords contain large amounts of glycogen and appear to be major areas of glycogen deposition. Fairbairn and Passey (1957) reported that in some nematodes, the concentration of glycogen and trehalose reaches 70% of the dry weight of the body wall muscle and Harpur (1962) reported 16% glycogen in fresh muscle tissue of nematodes. According to von Brand (1966), glycogen is located primarily in the muscles and lateral lines of nematodes. Lipids appear to be present in small amounts in the body wall musculature of M. subnigrescens and a slight reaction occurs in the hypodermal invaginations. It could not be determined if the lipids were concentrated in a particular area of the muscle or were diffused throughout the muscle. Von Brand (1966) reported that the plasma bulbs (non-contractile portion of the muscle cell) in body wall musculature are the most important storage areas for lipids in

nematodes.

The trophosomal wall seems to be highly reactive to various protein, carbohydrate, and lipid stains. This may indicate that this is a region of high metabolic activity and may be concerned, along with the hypodermal chords, with the formation of and/or metabolic breakdown of trophosomal spheres.

There has long been a controversy over the chemical composition of the trophosomal spheres. German workers (Meissner, 1854; von Linstow, 1898) have referred to the entire trophosomal mass as the "Fettkorper" inferring that the storage material was lipid. Chitwood and Jacobs (1938), working on Agamermis decaudata and using many of the above tests for lipids and proteins, concluded that stored nutritive materials in this species are of two types, namely "...a protein with reactions of a conjugated fatty acid-protein, and a neutral fat." This present study on M. subnigrescens tends to lead to a similar conclusion.

Since the histochemical study of the postparasitic juvenile was substantially the same as for the five week parasitic juvenile, the conclusions given above generally apply to both juvenile stages.

No completely satisfactory explanation can be given as to why there are apparently fewer proteins and free amino acids present in the parasitic juvenile as compared to the postparasitic juvenile. One possible explanation may be that the protein and/or specific amino acids were depleted in the 32-35 day-old parasitic juvenile due to the recent formation of the integument. The integument undergoes a rapid increase in thickness between the 28th and 38th day. Further studies on younger parasitic juveniles may substantiate this supposition.

PRELIMINARY INVESTIGATIONS ON THE FINE STRUCTURE OF
MERMIS SUBNIGRESCENS

Integument

Chitwood and Chitwood (1937) described the integument of Mermis subnigrescens as consisting of five distinct layers, namely: cortical layer, two fiber layers, matrix, and basal lamella. They observed that the cortical layer is "subdivisible" but that each division is apparently homogeneous. According to them, the fiber layers are bordered "externally, medially, and internally" by areas of dense matrix. The matrix was described as being multilayered and bordered internally by a delicate basal lamella.

In the present study, the integument appeared approximately 28 microns thick. The cortical layer, 0.5 microns thick, is divided into an outer portion 0.05 microns thick, and a less electron dense inner region 0.45 microns thick (Figure 47). Both layers lack any defined structures.

The two fiber layers are separated from the cortical layer by a finely granular osmiophilic layer approximately 0.8 microns in thickness. A similar layer, averaging 0.5 microns in thickness, separates the two fiber layers from one another. The outer, highly osmiophilic fiber layer measures 2.1 microns; the inner, less osmiophilic layer is 2.4 microns thick.

The thickest layer, the matrix, is 19 microns thick. In cross sections, this area appears as a spongy mass in which are found numerous fibers running lengthwise in the integument. Such fibers form a loose

arrangement of concentric rings when cross sections are viewed by light microscopy. Since the matrix stained heavily for collagen with Van Giesen's stain, these fibers are assumed to be collagen.

The innermost layer, the basal lamella, is 2.4 microns in width (Figure 48). This layer is composed of loosely scattered bodies within which appear numerous tightly packed fibrils. Possibly, these represent the incipient collagen fibers seen in the matrix, since these bodies become somewhat more abundant in the region of the matrix.

Hypodermis

Lying between the basal lamella and body wall musculature is the hypodermis, approximately 2 microns thick. At the interface of the basal lamella and the hypodermis, are numerous folds, the plasma membrane folds similar to those described by Rosenbluth (1965) in Ascaris lumbricoides. Numerous glycogen rosettes (Fawcett, 1966) are scattered throughout the layer (Figure 50). Histochemical evidence indicates that the hypodermis is rich in glycogen. Endoplasmic reticulum, lined with ribosomes, is abundant. Vacuolated areas, averaging over 0.3 microns in diameter, are numerous in the outer half of the layer, but less abundant internally as one nears the musculature. Small bodies, similar to those found in the basal lamella and presumed to contain incipient collagen, are also numerous throughout this layer (Figures 49 and 50).

Body Wall Musculature

Muscles of the body wall lie below the hypodermal layer and are separated from it by a basement membrane. Individual myofibers are ap-

proximately 1.5 to 1.8 microns wide in cross section.

Apparently two types of myofibers are present (Figure 51). In one type, individual myofilaments are dense and their arrangement is characteristic of "fast" muscle. A second type of myofiber, characteristic of "slow" muscle, possesses thicker myofilaments having a greater diameter than those of "fast" muscle.¹

Sarcoplasmic reticulum is well developed (Figure 51) in each type of myofiber. Projecting into each myofiber for a distance of 0.6 to 0.7 microns from the periphery are thickened areas. These areas appear to be regularly spaced, usually 1.2 to 1.5 microns apart. They are in the same position as the Z bands in obliquely cut myofibers of Ascaris lumbricoides, as described by Rosenbluth (1965). Myofilaments around these projections appear to be exclusively thin myofilaments and apparently represent the I bands in cross section as described by Rosenbluth (1965).

Trophosomal Spheres

Structurally, larger and smaller trophosomal spheres are apparently similar, although histochemically, as indicated above, they differ from one another. Their apparent similarity may be due to the method of fixation used in this study. The material within the spheres appears to be homogeneous.

¹This interpretation of micrographs dealing with muscle is based on comments made to me by Mr. Ross Johnson, Department of Zoology, Iowa State University, Ames, Iowa.

Material outside and surrounding the spheres seems to consist of aggregates of small granules. Since this area stained strongly for glycogen, it can be assumed that such aggregates represent glycogen rosettes. Also present are larger granules interspersed among the glycogen (Figure 52). These may be deposits of lipid since this area between the spheres has a strong affinity for lipid stains.

Esophageal Apparatus

The esophageal wall is heavily osmiophilic (Figure 53). This affinity for osmium masks whatever structures are present, if any, and makes interpretation of this area difficult.

Numerous fine, fibrous extensions project into the tissue of the esophageal sheath from the outer portion of the esophageal wall. The exact nature of these fibers is not known. The matrix-like sheath surrounding the esophageal wall is composed of numerous small fibrils lying in all directions. As shown in Figure 53, the presence of a Golgi apparatus near the esophageal wall gives added evidence that the esophageal apparatus is of a secretory nature, as observed by Chitwood (1935) and by Baylis (1947).

Numerous small mitochondria are present, as are bodies similar to those thought to contain incipient collagen fibers observed in the hypodermal and basal lamella layers.

SUMMARY AND CONCLUSIONS

1. Mermis subnigrescens, a nematode whose juveniles parasitize insects of the Order Orthoptera, was investigated as to morphology of parasitic juveniles, histochemistry of parasitic and postparasitic stages, host-parasite relationships, bionomics of the various life cycle stages and fine structure of the body wall and certain internal body parts.

2. M. subnigrescens eggs, ingested by grasshoppers, hatch in the host's alimentary canal. After escape of the juvenile from the egg, it bores through the intestinal wall and begins its parasitic development within the haemocoel. Four to ten weeks later, the juvenile emerges from the host and completes its development in the soil.

3. The first postparasitic moult occurs approximately two months after emergence, under laboratory conditions, and eggs are first laid approximately twenty months after emergence.

5. Although gravid females lay more eggs in the presence of sunlight, oviposition may occur in total darkness. The presence of haemoglobin, previously thought to be associated with egg laying, is probably concerned with respiration.

6. The average annual mermithid infection in grasshopper populations in central Iowa is approximately 16 to 17 per cent.

7. A slightly higher percentage of female grasshoppers is infected with mermithids than are males.

8. M. subnigrescens apparently infects only members of the families Acrididae and Tettigoniidae in central Iowa. Attempts to infect members

of the family Gryllidae were unsuccessful.

9. A host list of experimental and/or natural infections of M. subnigrescens indicates that at least 15 species of grasshoppers of central Iowa may harbor this parasite.

10. Grasshoppers, once infected with M. subnigrescens, are not refractile to subsequent infections, although the initial infection seems to appear to be heavier than subsequent infections.

11. Growth rate of M. subnigrescens and M. nigrescens juveniles were compared. Juveniles of M. subnigrescens appear to grow at a somewhat faster rate than do those of M. nigrescens of a similar age.

12. The early juvenile stages are described in detail during the first fourteen days of their parasitic development.

13. The body length of the parasite is probably influenced by both the size of the grasshopper host and the number of parasites present within the host. Different species of grasshoppers seem to have only a minimal effect on juvenile size.

14. Male juveniles remain in their host from four to six weeks; females from eight to ten weeks before emergence.

15. If less than five or six parasitic juveniles are present in a host, females predominate; in infections of from five to fifteen juveniles, most parasites are females but one or more males may be present; and in infections of over 20 juveniles, male parasites predominate.

16. Description of organ formation and certain effects of the parasitic juvenile on the host are presented. Host damage appears to be due principally to mechanical damage by the juvenile.

17. Toxic products from the worm may be a factor in the reduction of the number of eggs produced by the host. These toxic products, along with mechanical damage and nutrient depletion, may all be factors limiting host egg production.

18. Data obtained in this study corroborate the viewpoint that M. subnigrescens is a distinct and separate species.

19. Histochemical tests indicate that the integument is composed of a variety of substances, but that apparently no lipids are present. The hypodermis is rich in glycogen and lipids. Body wall musculature gave strong reactions to protein, carbohydrates, especially glycogen, and, to a lesser degree, lipids. The large trophosomal spheres appear to be composed entirely of protein; The smaller spheres, of a protein-lipid complex.

20. Parasitic and postparasitic stages were compared histochemically and both stages appear essentially similar. There appears to be less protein and/or specific amino acids present in the parasitic juvenile. This may be due to their incorporation into the greatly thickened integument which develops shortly before emergence of the juveniles.

21. Data on preliminary investigations on fine structure of the integument, hypodermis, body wall musculature, trophosomal spheres, and esophageal apparatus of postparasitic juveniles are presented.

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APPENDIX

List of North American Hosts of Mermithids

Mermis subnigrescens

Orthoptera

Family Acrididae	Reference
<u>Arphia sulphurea</u> Fabricius	Christie, 1937
<u>Camnula pellucida</u> Scudder	Christie, 1937; Briand and Rivard, 1964
<u>Chorthippus curtispennis</u> Harris	Cobb, 1926a
<u>C. longispennis</u> Latreille	Cobb, 1926a
<u>Chortophaga viridifasciata</u> DeGeer	Cobb, 1926a; Briand and Rivard, 1964; Christie, 1937
<u>Encoptolophus sordidus</u> Burmeister	Cobb, 1926a; Christie, 1937
<u>Melanoplus atlantis</u> (= <u>mexicanus mexicanus</u>) Riley	Christie, 1937; Briand and Rivard, 1964
<u>M. bivittatus</u> Say	Christie, 1937; Briand and Rivard, 1964
<u>M. differentialis</u> Thomas	Hayes and DeCoursey, 1938
<u>M. femurrubrum</u> DeGeer	Cobb, 1926a; Hayes and DeCoursey, 1938; Christie, 1937; Briand and Rivard, 1964
<u>M. luridus</u> Dodge	Briand and Rivard, 1964
<u>M. mexicanus</u> (?) Saussure	Christie, 1937
<u>Orphulella pelidna</u> Burmeister	Cobb, 1926a; Christie, 1937
<u>O. speciosa</u> Scudder	Briand and Rivard, 1964
<u>Romalea microptera</u> Palisot de Beauvois	Christie, 1937
<u>Spharagemon collare</u> Scudder	Briand and Rivard, 1964

Family Tettigoniidae	Reference
<u>Conocephalus brevipennis</u> Scudder	Christie, 1937; Briand and Rivard, 1964
<u>C. fasciatus</u> DeGeer	Briand and Rivard, 1964
Family Gryllacrididae	
<u>Stenopelmatus lonhidpins</u>	Cobb, 1926a
Hymenoptera	
Family Aphididae	
<u>Apis mellifera</u> Linnaeus	Milum, 1938
<u>Agamermis decaudata</u>	
Orthoptera	
Family Acrididae	
<u>Camnula pellucida</u> Scudder	Smith, 1944
<u>Dissosteira carolina</u> Linnaeus	Walton, 1927
<u>Melanoplus bivittatus</u> Say	Smith, 1944; Pickett, 1935
<u>M. femoratus</u> Burmeister	Christie, 1937
<u>M. femurrubrum</u> DeGeer	Christie, 1937
<u>M. mexicanus</u> (?) Saussure	Christie, 1937; Smith, 1944; Pickett, 1935
<u>M. sanguinipes</u>	Smith, 1965
Family Tettigoniidae	
<u>Orchelimum vulgare</u> Harris	Christie, 1937; Cobb, Steiner, Christie, 1923

Homoptera

Family Cicadellidae

Draeculacephala mollipes Say Christie, 1936

Araneida (Araneae)

Phidippus clarus Keyserling Kaston, 1945

Mermis nigrescens

Orthoptera

Family Acrididae

Camnula pellucida Scudder Painter, Fluke, and Granovsky,
1925; Granovsky, 1926

Chortophaga viridifasciata DeGeer Smith, 1965

Dissosteira carolina Linnaeus Granovsky, 1926

Melanoplus atlantis (= mexicanus
mexicanus) Riley Granovsky, 1926

M. bivittatus Scudder Granovsky, 1926

M. femurrubrum DeGeer Granovsky, 1926

M. luridus Dodge Granovsky, 1926

M. sanguinipes Say Smith, 1965

Mermis albicans

Orthoptera

Family Acrididae

Dissosteira carolina Linnaeus Leidy, 1856; Chittenden, 1905

Locusta (= Dissosteira) carolina Leidy, 1851

Melanoplus atlantis Riley Glaser and Wilcox, 1918

M. differentialis Thomas Riley, 1878; Chittenden, 1905

Family Tettigoniidae

Orchelimum gracile Harris Leidy, 1856

Lepidoptera

Family Olethreutidae

Carpocapsa pomonella Linnaeus Chittenden, 1905

Mermis ferruginea

Orthoptera

Family Acrididae

Dissosteira carolina Linnaeus Glaser and Wilcox, 1918

Melanoplus atlantis Riley Glaser and Wilcox, 1918

Mermis acuminata

Orthoptera

Family Acrididae

Schistocerca americana Drury Riley, 1878

Hexameris arvalis

Coleoptera

Family Curculionidae

Hypera postica Gyllenhal Poinar and Gyrisco, 1962

H. variabilis Herbst Poinar and Gyrisco, 1962

Hexameris microamphidis

Lepidoptera

Family Crambidae

Diatraea saccharalis Fabricius Jaynes, 1933

Hexameris sp.

Orthoptera

Family Acrididae

Chortophaga viridifasciata DeGeer Blickenstaff and Sharifullah, 1962

Hemiptera

Family Miridae

Lygus pratensis (= lineolaris) Painter, 1929
Linnaeus

Coleoptera

Family Curculionidae

Pantomorus peregrinus Buchanan Swain, 1943

P. leucoloma Boheman Swain, 1943

Family Cerambycidae

Monochamus scutellatus Say Soper and Olson, 1963

Diptera

Family Anthomyiidae

Hylemya antiqua Meigen Flint and Dungan, 1928

Araneida (Araneae)

Xysticus funestus Keyserling Kaston, 1945

Orthomeris oedobanchus

Coleoptera

Family Staphylinidae

Omalium caesum Gravenhorst Poinar, 1964

Diptera

Family Chironomidae

Smittia aterrima Meigen

Poinar, 1964

Family Sciaridae

Lycoriella solani Frey

Poinar, 1964

Amphimermis bogongae

Lepidoptera

Family Yponomeutidae

Agrotis infusa Boisduval

Welch, 1963

Hydromermis churchillensis

Diptera

Family Chaoboridae

Chaoborus sp.

Welch, 1960

Mochlonyx sp.

Welch, 1960

Family Culicidae

Aedes communis DeGeer

Welch, 1960; Smith, 1961

Aedes pullatus Coquillett

Smith, 1961

Hydromermis spp.

Diptera

Family Culicidae

Aedes cummunis DeGeer

Jenkins and West, 1954

A. nearcticus Dyar

Jenkins and West, 1954

Family Simuliidae

Cnephia sp. Enderlein

Anderson and Dicke, 1960

<u>Eusimulium</u> sp. Roubaud	Anderson and Dicke, 1960
<u>Prosimulium</u> sp.	Anderson and Dicke, 1960
<u>Prosimulium exigens</u>	Peterson, 1960
<u>Simulium</u> sp.	Anderson and Dicke, 1960
<u>Simulium articum</u> Malloch	Peterson, 1960

Hydromermis conopophaga

Diptera

Family Chironomidae

<u>Tanytarsus</u> sp.	Poinar, 1960
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Hydromermis contorta

Diptera

Family Chironomidae

<u>Chironomus plumosus</u> Linnaeus	Johnson, 1955
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Family Simuliidae

<u>Simulium tuberosum</u> Lundström	Johnson, 1955
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Paramermis canadensis

Diptera

Family Culicidae

<u>Aedes vexans</u> Meigen	Steiner, 1924
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Pseudomermis zykoffi

Diptera

Family Chironomidae

<u>Chironomus</u> sp.	Cobb, Steiner, and Christie, 1927
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Gastromermis viridis

Diptera

Family Simuliidae

<u>Simulium vittatum</u> Zetterstedt	Phelps, 1962; Phelps and DeFoliart, 1964; Welch, 1962
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Gastromermis app.

Diptera

Family Simuliidae

<u>Cnephia mutata</u> DeFoliart	Anderson and DeFoliart, 1962
<u>Prosimulium fuscum</u> Syme and Davis	Anderson and DeFoliart, 1962
<u>P. magnum</u> Dyar and Shannon	Anderson and DeFoliart, 1962
<u>Simulium corbis</u> Twinn	Anderson and DeFoliart, 1962
<u>S. decorum</u> Walker	Anderson and DeFoliart, 1962
<u>S. jenningsi</u> Malloch	Anderson and DeFoliart, 1962
<u>S. venustum</u> Say	Anderson and DeFoliart, 1962
<u>S. vittatum</u> Zetterstedt	Anderson and DeFoliart, 1962

Isomermis wisconsinensis

Diptera

Family Simuliidae

<u>Simulium vittatum</u> Zetterstedt	Phelps and DeFoliart, 1964; Welch, 1962
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Isomermis spp.

Family Simuliidae

<u>Cnephia emergens</u> Shewell	Anderson and DeFoliart, 1962
<u>C. mutata</u> DeFoliart	Anderson and DeFoliart, 1962
<u>Eusimulium latipes</u> Meigen	Anderson and DeFoliart, 1962

<u>Prosimulium fuscum</u> Syme and Davis	Anderson and DeFoliart, 1962
<u>P. magnum</u> Dyar and Shannon	Anderson and DeFoliart, 1962
<u>Simulium decorum</u> Walker	Anderson and DeFoliart, 1962
<u>S. luggeri</u> Walker	Anderson and DeFoliart, 1962
<u>S. tuberosum</u> Lundstrom	Anderson and DeFoliart, 1962
<u>S. venustum</u> Say	Anderson and DeFoliart, 1962
<u>S. vittatum</u>	Anderson and DeFoliart, 1962

Mesomermis fluminalis

Diptera

Family Simuliidae

<u>Cnephia mutata</u> DeFoliart	Phelps and DeFoliart, 1964
<u>Prosimulium fuscum</u> Syme and Davis	Phelps and DeFoliart, 1964
<u>P. magnum</u> Dyar and Shannon	Phelps and DeFoliart, 1964
<u>Simulium venustum</u> Say	Welch, 1962; Phelps and DeFoliart, 1964; Phelps, 1962

Tetradonema plicans

Diptera

Family Sciaridae

<u>Sciara coprophila</u> Lintner	Cobb, 1919
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Mermithonema entomophilum

Diptera

Family Sepsidae

<u>Sepsis cynipsea</u> Linnaeus	Goodey, 1941
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Nanomermis nemicola

Nematoda

Family Mononchidae

Mononchus subtenuis Chitwood Cobb, 1925Agamomermis infestans

Coleoptera

Family Silphidae

Nicrophorus sp. Christie, 1930Agamomermis culicis

Diptera

Family Culicidae

Aedes vexans Meigen Stabler, 1945, Welch, 1960Anopheles sp. Welch, 1960Culex pipiens Linnaeus Welch 1960, Stabler, 1951C. salinarius Coquillett Welch, 1960Agamomermis pachysoma

Hymenoptera

Family Vespidae

Vespula germanica Fabricius Welch, 1958V. vulgaris Welch, 1958Agamomermis spp.

Family Sialidae

Sialis lutaria Linnaeus Stiles, 1903

Diptera

Family Culicidae

Aedes nemorosus Meigen Stiles, 1903

<u>A. sollicitans</u> Walker	Stiles, 1903
<u>Culex memorialis</u>	Stiles, 1903
<u>C. pipiens</u> Linnaeus	Stabler, 1951
<u>C. salinarius</u> Coquillett	Stabler, 1945

Unidentified Memithids

Collembola

Family Poduridae

<u>Neanura grassei</u> Denis	Phillips, 1946
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Orthoptera

Family Acrididae

<u>Aeropedellus clavatus</u> Thomas	Smith, 1958
<u>Camnula pellucida</u> Scudder	Smith, 1944, 1958
<u>Chorthippus longicornis</u> Latreille	Smith, 1958
<u>Lucusta (=Dissosteira) carolina</u> Linnaeus	Glaser and Wilcox, 1918
<u>Melanoplus bivittatus</u> Say	Glaser and Wilcox, 1918
<u>M. dawsoni</u> Scudder	Smith, 1958
<u>M. femurrubrum</u> DeGeer	Smith, 1944, 1958, Tauber and Yeager, 1935
<u>M. gladstoni</u> Scudder	Smith, 1958
<u>M. infantilis</u> Scudder	Smith, 1958
<u>M. mexicanus</u> (?) Saussure	Smith, 1958
<u>M. packardii</u> Scudder	Smith, 1958

Family Tettigoniidae

<u>Conocephalus saltans</u> Scudder	Smith, 1958
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Family Gryllidae

Acheta assimilis Fabricius

Smith, 1958

Oecanthus sp. Serville

Parrott and Fulton, 1914

Isoptera

Family Termitidae

Cornitermes enthocephalus Silverman

Ruttledge, 1925

Homoptera

Family Aphididae

Anoecia sp.

Davis, 1916

Family Curculionidae

Conotrachelus aratus Germain

Brooks, 1922

Coleoptera

Family Scarabaeidae

Lachnosterna (=Phyllophaga)

McColloch and Hayes, 1923

Phyllophaga "grubs"

Davis, 1919

Phyllophaga anxia LeConte

Criddle, 1918

P. drakii Kerby

Criddle, 1918

P. nitido Linnaeus

Criddle, 1918

P. rugosa Melsheimer

Criddle, 1918

Family Scolytidae

Dendroctonus monticolae Hopkins

Thorne, 1935

Lepidoptera

Family Tortricidae

Carpocarpa pomonella Linnaeus

Brooks and Blakeslee, 1915

Family Noctuidae

Papaipema nitela Guenee

Lowery, 1927

Diptera

Family Culicidae

<u>Aedes nigripes</u> Zetterstedt	Jobbins-Pomeroy, 1916
<u>A. vexans</u> Meigen	Laffoon, 1951

Family Ceratopogonidae

<u>Culicoides crepuscularis</u> Malloch	Smith, 1958
<u>C. haematopodus</u> Malloch	Smith, 1958
<u>C. stellifer</u> Coquillett	Smith, 1958
<u>Leptoconops kerteszi</u> Kieffer	Whitsel, 1965

Family Simuliidae

<u>Cnephia mutata</u> Malloch	Anderson and DeFoliart, 1962
<u>Eusimulium aureum</u> Fries	Anderson and DeFoliart, 1962
<u>Gyanopais</u> sp.	Sommerman, Sailer, and Esselbough, 1955
<u>Prosimulium</u> sp.	Sommerman, Sailer, and Esselbough, 1955
<u>P. cecemarticulatum</u>	Anderson and DeFoliart, 1962
<u>P. fuscum</u> Syme and Davis	Davis, 1958
<u>P. gibsoni</u> Twinn	Anderson and DeFoliart, 1962
<u>P. hirtipes</u> Fries	Wu, 1931
<u>P. magnum</u> Dyar and Shannon	Anderson and DeFoliart, 1962
<u>P. mixtum</u> Syme and Davis	Anderson and DeFoliart, 1962 Davis, 1958
<u>Simulium</u> sp.	Jobbins-Pomeroy, 1916
<u>Simulium</u> sp. larvae	Jobbins-Pomeroy, 1916
<u>S. bracteatum</u> Coquillett	Strickland, 1913
<u>S. decorum</u> Walker	Anderson and DeFoliart, 1962

<u>S. multidentatum</u> Twinn	Twinn, 1939
<u>S. tuberosum</u> Lundström	Anderson and DeFoliart, 1962
<u>S. venustum</u> Say	Hocking and Pickering, 1954; O'Kane, 1926; Jobbins-Pomeroy, 1916, Twinn, 1939, Anderson and DeFoliart, 1962
<u>S. vittatum</u> Zetterstedt	Anderson and DeFoliart, 1962

Family Chironomidae

<u>Chironomus lobiferus</u> Say	Johnson, 1956
<u>C. palliatus</u> Coquillett	Johnson, 1956
<u>C. tentans</u> Fabricius	Sadler, 1935
<u>C. viridis</u> Malloch	Johnson, 1956

Hymenoptera

Family Vespidae

<u>Vespa vulgaris</u> Linnaeus	Beck, 1937
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Family Formicidae

<u>Acanthomyops flavus</u> Fabricius	Taylor, 1933
<u>Camponotus abdominalis</u>	Taylor, 1933
<u>Camponotus claripes</u>	Taylor, 1933
<u>Camponotus consobrinus</u>	Taylor, 1933

Homoptera

Family Aphididae

<u>Selandria serva</u>	Benson, 1926
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PLATES

Abbreviations Used

a	genital anlage	in	intestine	te	testis
ac	anterior cell	iw	intestinal wall	ts	trophosomal sphere
an	antenna	l	lateral oviduct	tw	trophosomal wall
b	byssus	lh	lateral hypodermal chord	u	uterus
bl	basal lamella	lm	longitudinal muscle	v	vulva
bm	body wall musculature	m	muscle cell	va	vagina
c	compound eye	ml	matrix layer	vc	ventral nerve cord
d	deutocerebrum	n	neurilemma	vh	ventral hypodermal chord
dh	dorsal hypodermis	nm	nerve mass		
e	egg	o	outer membrane		
ea	esophageal apparatus	oc	outer cortical layer		
er	endoplasmic reticulum	ol	osmiophilic layer		
ew	esophageal wall	p	parasitic juvenile		
f	fracture line	pm	plasma membrane fold		
fb	fat body	pr	protocerebrum		
fl	fiber layer	s	stylet		
fm	"fast" myofiber	sc	stichocyte		
g	glycogen rosette	sm	"slow" myofiber		
ga	Golgi apparatus	sg	salivary gland		
h	hypodermal layer	sr	sarcoplasmic reticulum		
hc	hypodermal chord	st	stichocyte rudiment		
i	inner membrane	t	trophosome		
ic	inner cortical layer				

Plate I

- Figure 1. Egg of Mermis subnigrescens showing byssus and fracture line.
- Figure 2. Cross section of gravid female of M. subnigrescens.
Approximately 1080X.
- Figure 3. Newly emerged parasitic juvenile in haemocoel of grasshopper (Melanoplus sp.) showing penetration route through grasshopper intestinal wall. Approximately 1150X.
- Figure 4. Anterior end of newly emerged parasitic juvenile.

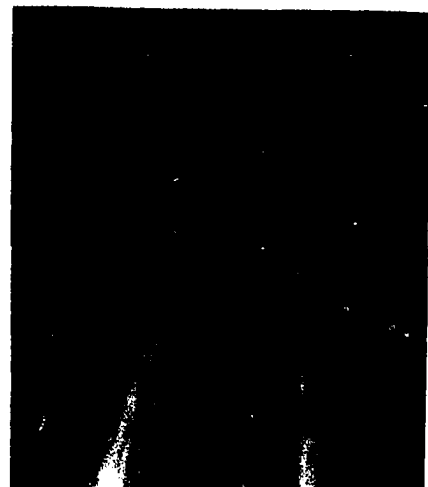
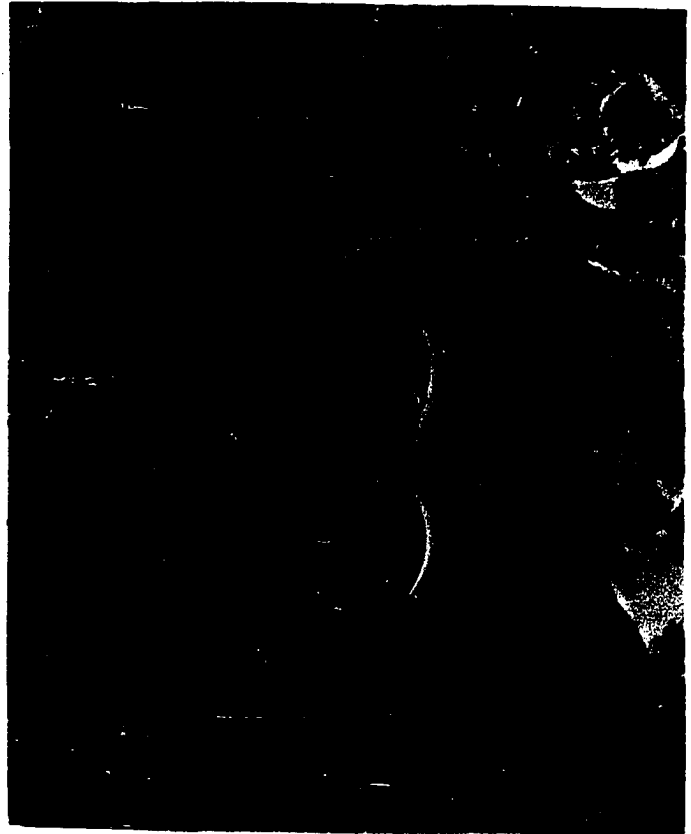


Plate II

- Figure 5. Parasitic juvenile of M. subnigrescens. Cross section showing vase-shaped muscle cells. Approximately 1120X.
- Figure 6. Cross section of parasitic juvenile showing early genital anlage. Approximately 430X.
- Figure 7. Cross section of parasitic juvenile showing later genital anlage. Approximately 1220X.
- Figure 8. Gravid female juvenile showing vulva, uterus, and eggs.

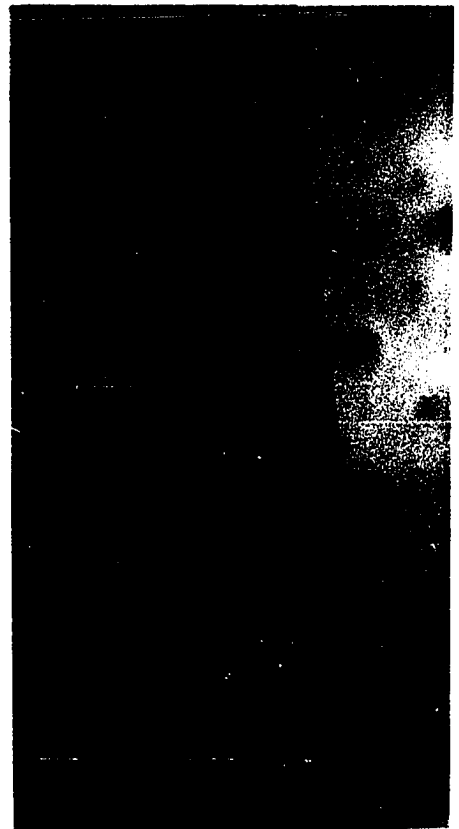
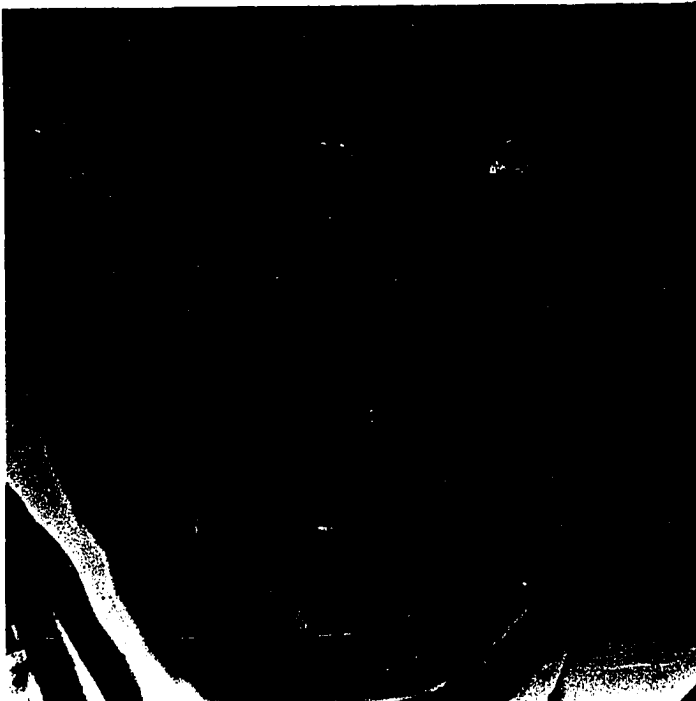
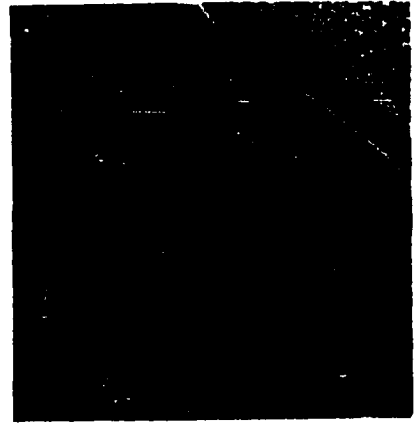
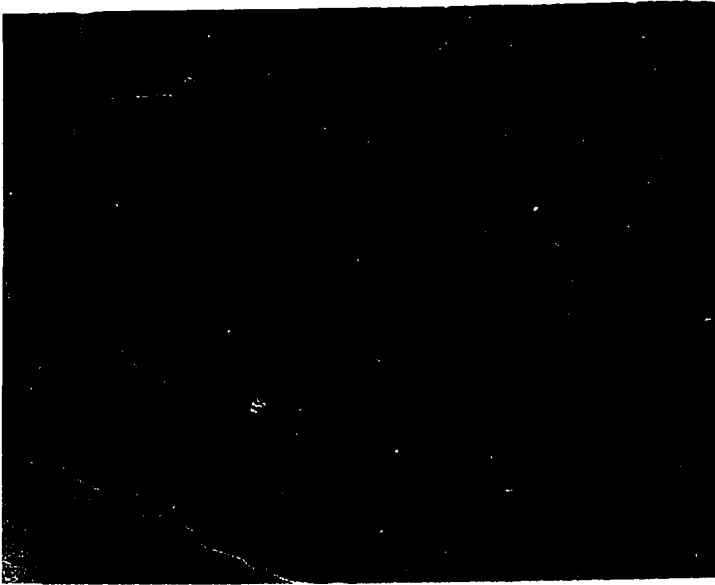


Plate III

- Figure 9. Parasitic juvenile of M. subnigrescens in mandibular adductor muscle of grasshopper. Approximately 860X.
- Figure 10. Longitudinal section through brain of non-parasitized grasshopper to show normal anatomical relationships. Approximately 480X.
- Figures 11 and 12. Longitudinal section of grasshopper brain showing parasitic juvenile in the area between protocerebrum and deutocerebrum. Approximately 430X.

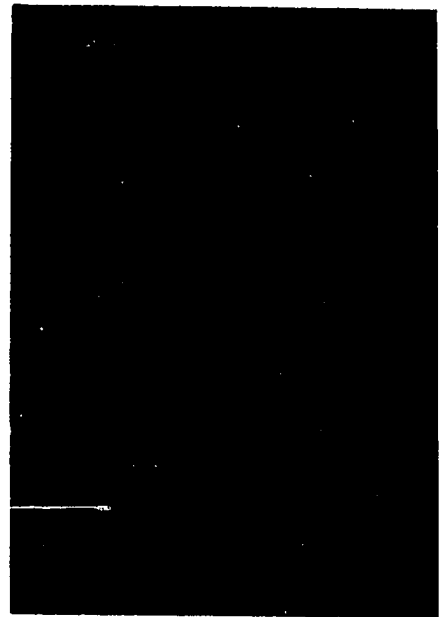
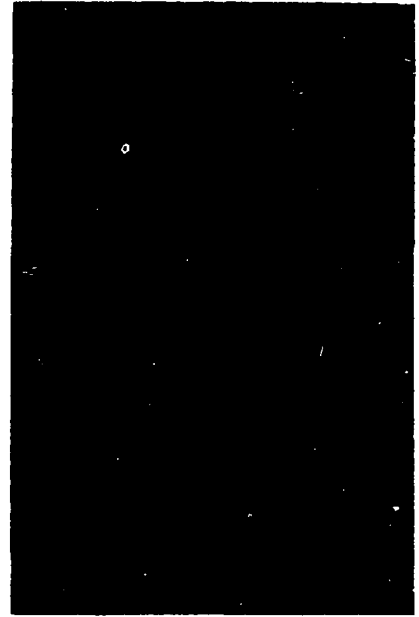


Plate IV

Figures 13 and 14. Longitudinal section through region of salivary glands of grasshopper parasitized by M. subnigrescens. Approximately 850X.

Figure 15. Normal salivary gland of grasshopper (Melanoplus sp.). Approximately 850 X.

Figure 16. Parasitic juvenile in body wall musculature of parasitized grasshopper. Approximately 780X.

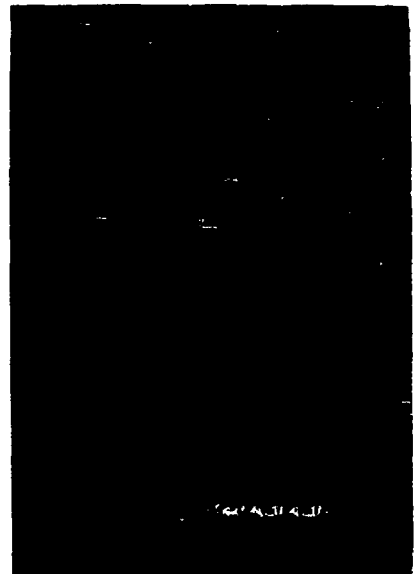
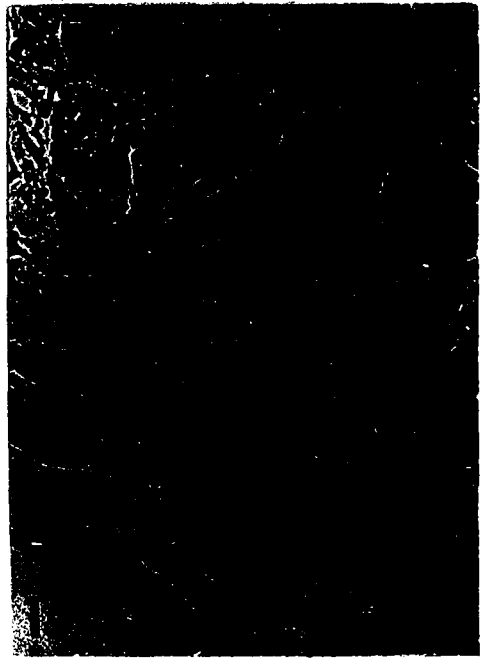


Plate V

Figure 17. Cross section through parasitized grasshopper showing reduced intestinal musculature due to the presence of parasitic juvenile of M. subnigrescens.

Figures 18 and 19. Parasitic juvenile in ventral fat body of grasshopper, longitudinal section. Figure 18 approximately 430X; Figure 19 approximately 960X.

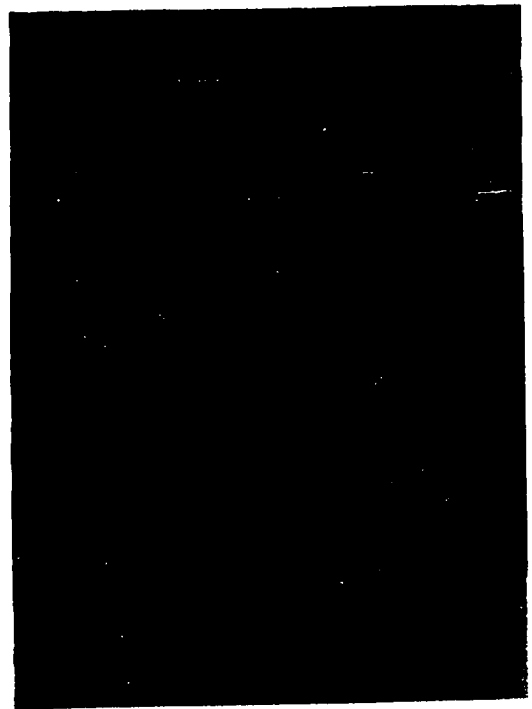
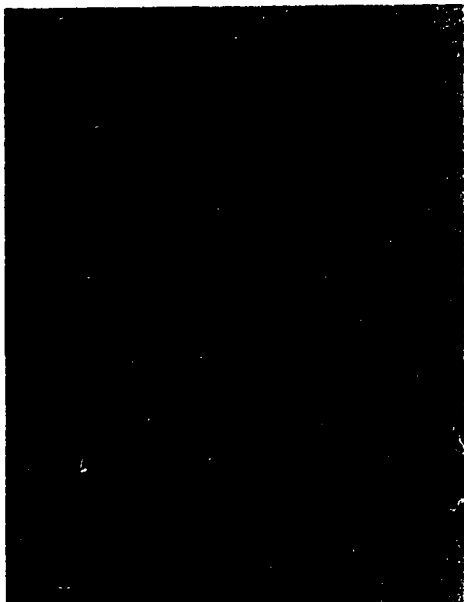


Plate VI

- Figure 20. Cross section of parasitized grasshopper showing closure of lateral oviduct against body wall. Approximately 430X.
- Figure 21. Cross section of a parasitized grasshopper showing closure of lateral oviduct against intestinal wall. Approximately 430X.
- Figure 22. Cross section through testes of a parasitized grasshopper. Approximately 430X.
- Figure 23. Parasitic juvenile of M. subnigrescens lying beneath the neurilemma of the grasshopper host. Approximately 430X.

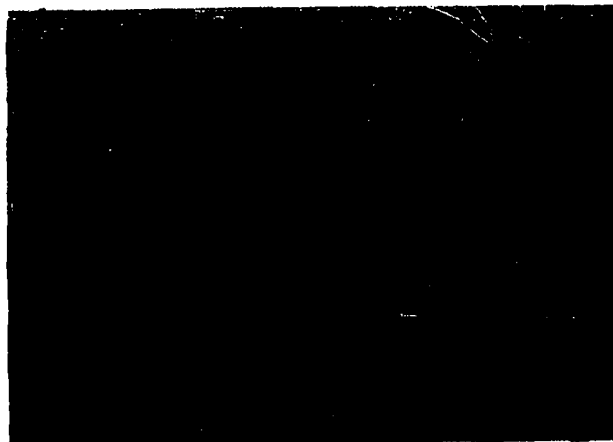
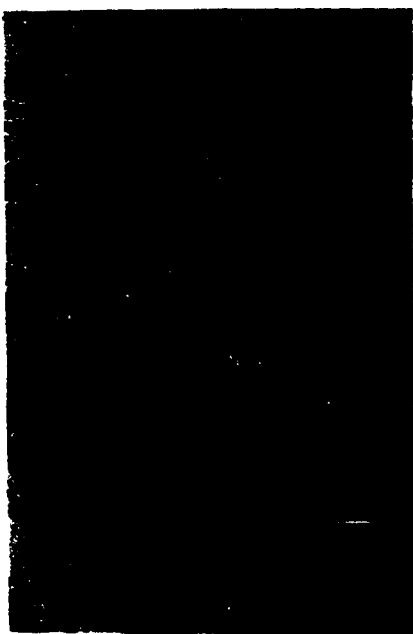
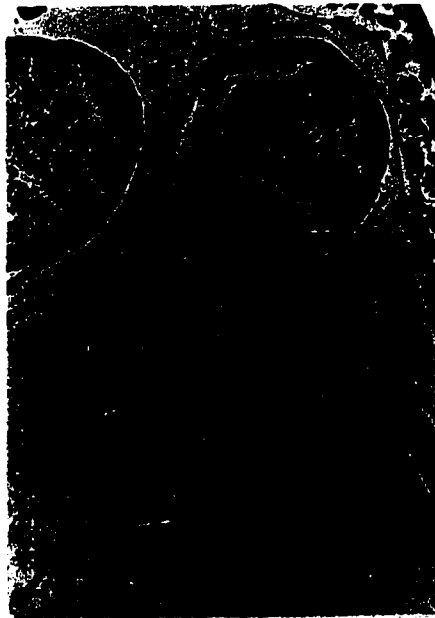


Plate VII

Figures 24, 25 and 26. Representative cross sections of adult Mermis subnigrescens.

Figure 24. Cross section approximately 15-18 microns from the anterior end. Approximately 650X.

Figure 25. Cross section showing processes from the longitudinal muscles going to the ventral nerve cord. Approximately 550X.

Figure 26. Cross section approximately three millimeters from the anterior end. Approximately 665X.



Plate VIII

Figure 27. Sakaguchi arginine reaction. Trophosomal spheres showing moderate to weak staining reaction. M. subnigrescens parasitic juvenile 30 days old. Approximately 560X.

Figures 28-30. Cross sections through formalin-fixed, recently emerged postparasitic juvenile M. subnigrescens.

Figure 28. Alcian blue stain for acid mucopolysaccharides. Approximately 560X.

Figure 29. Normal periodic acid-Schiff (PAS) reaction. Approximately 365X.

Figure 30. Control section for PAS reaction. Section not oxidized with periodic acid before staining. Approximately 560X.

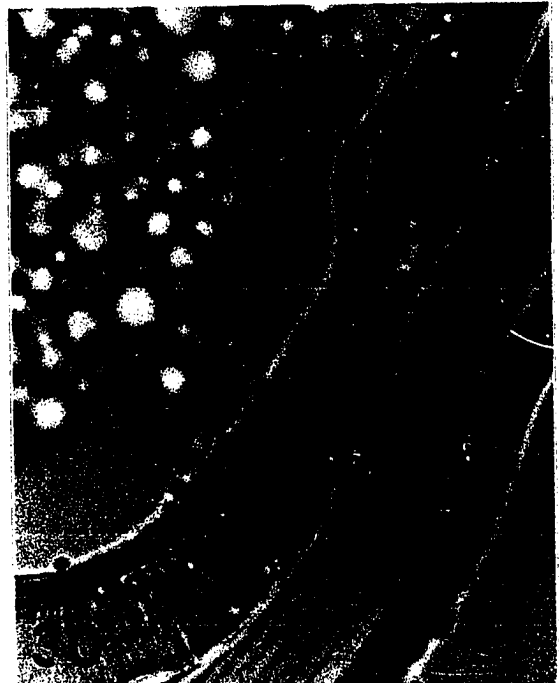
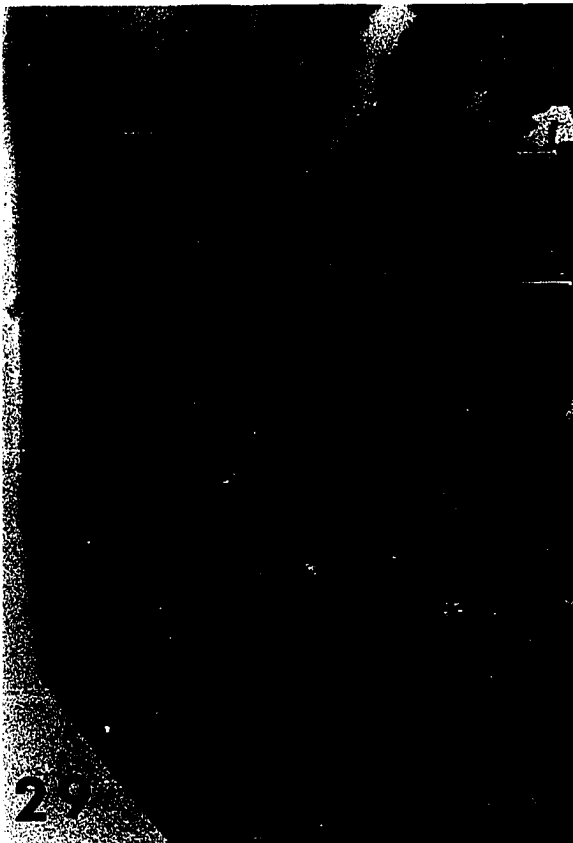
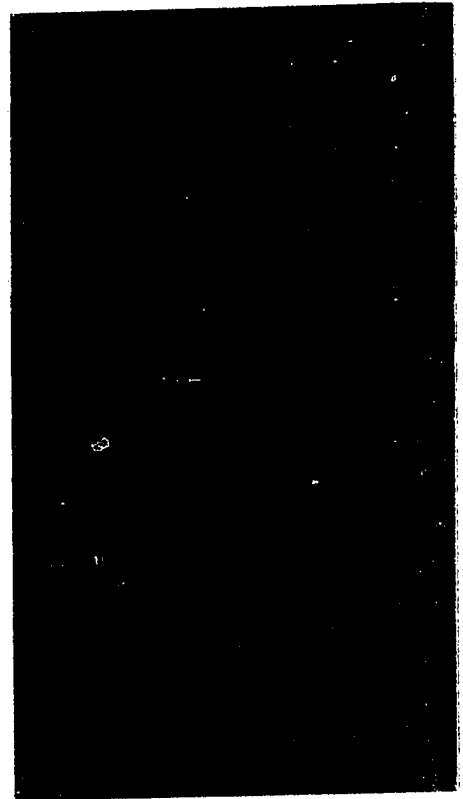
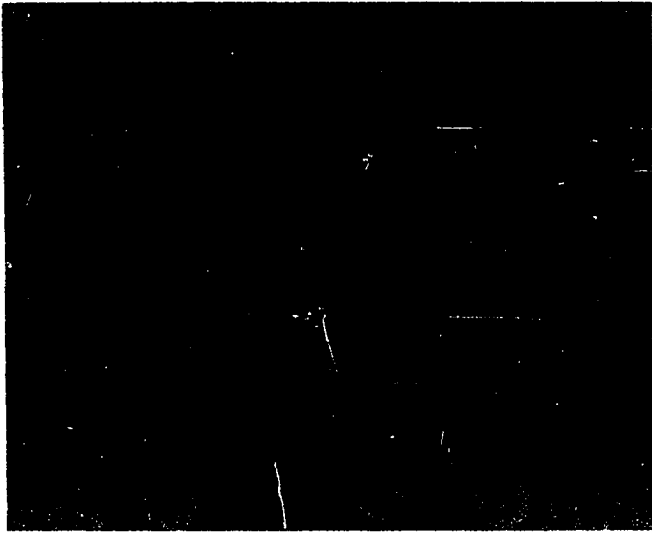


Plate IX

Figures 31-34. Cross sections through formalin fixed recently emerged postparasitic juvenile M. subnigrescens.

Figure 31. Periodic acid-Schiff (PAS) reaction after acetylation, cryostat preparation. Approximately 365X.

Figure 32. PAS reaction after acetylation, paraffin embedded preparation. Approximately 560X.

Figure 33. PAS reaction after deacetylation, cryostat preparation. Approximately 560X.

Figure 34. PAS reaction after deacetylation, paraffin embedded preparation. Approximately 560X.

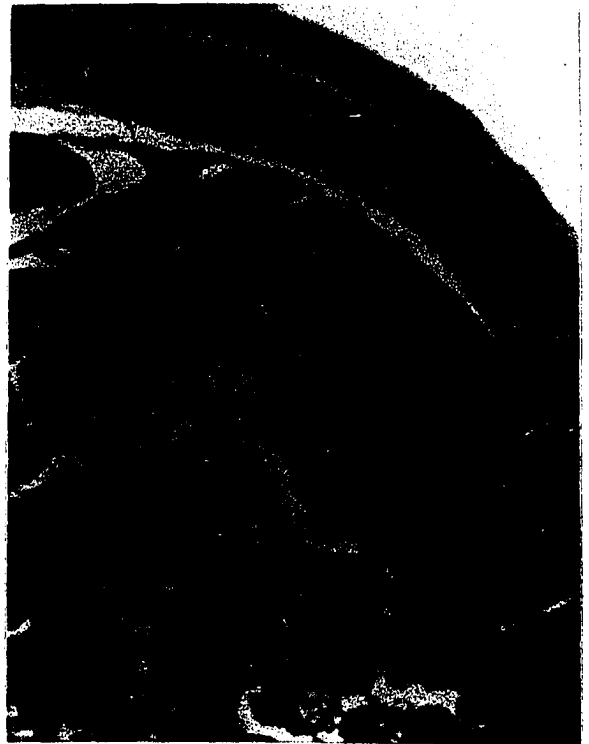
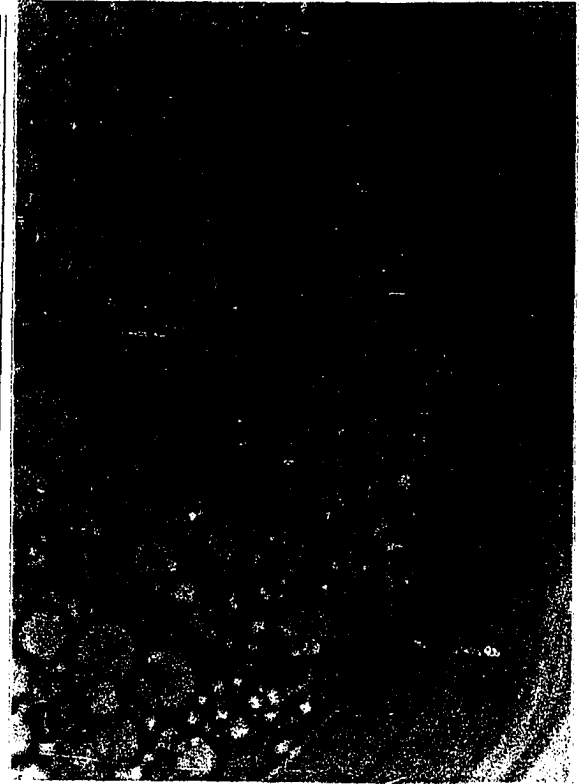
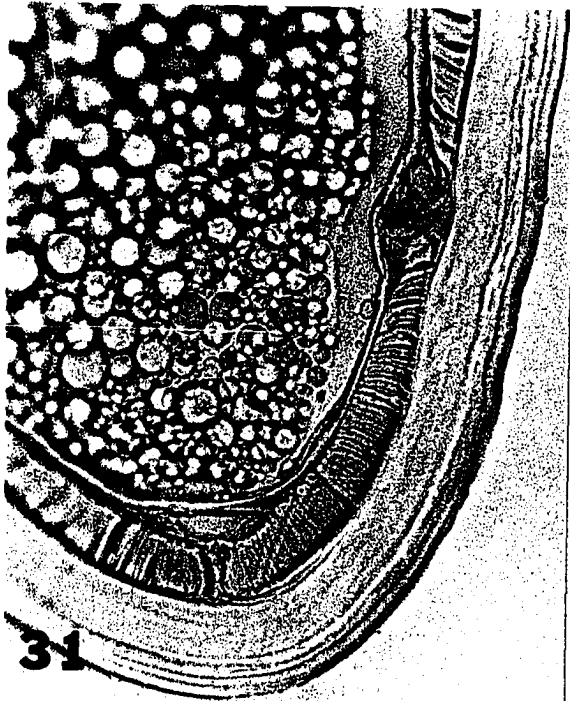


Plate X

Figures 35-38. Cross sections through juvenile M. subnigrescens, cryostat preparations stained for lipids.

Figure 35. Oil Red O, fresh frozen section of postparasitic juvenile. Approximately 560X.

Figure 36. Scharlach R, formalin fixed sections, 37 day old parasitic juvenile. Approximately 560X.

Figure 37. Teased section of postparasitic juvenile. Nile Blue S preparation. Neutral fats appear red; fatty acids, blue. Approximately 560X.

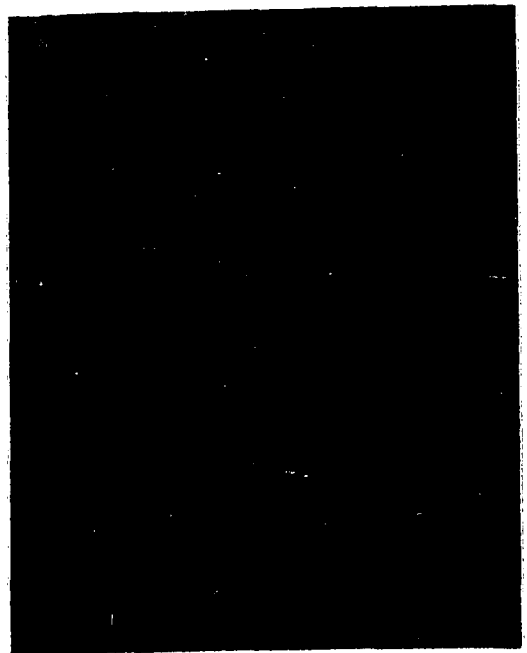
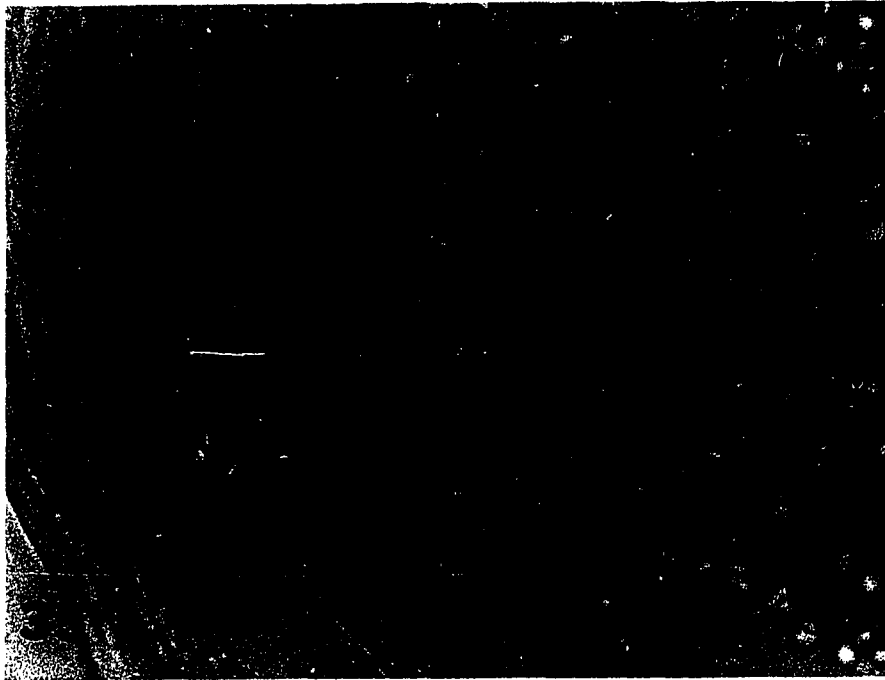


Plate XI

Figures 38-40. Cross sections through formalin fixed, cryostat preparations of parasitic juveniles of M. subnigrescens, stained for periodic acid-Schiff (PAS) reaction.

Figure 38. 34 day old juvenile. Normal PAS reaction. Approximately 365X.

Figure 39. 37 day old juvenile, control section (no periodic acid). Approximately 560X.

Figure 40. 37 day old juvenile, control section stained with PAS reaction after acetylation. Approximately 560X.

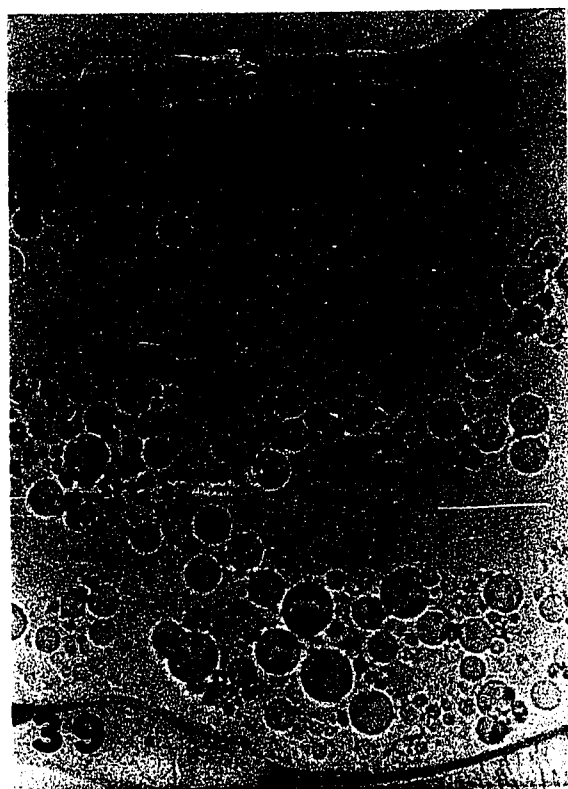
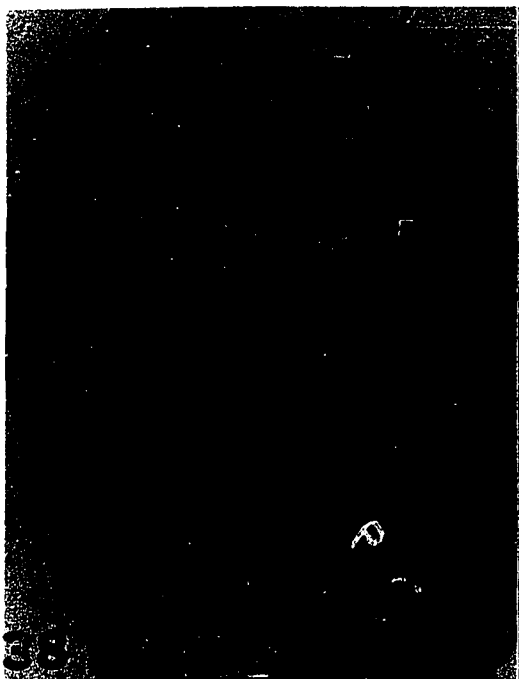


Plate XII

Figures 41-46. Developmental stages of parasitic juvenile Mermis subnigrescens from experimentally infected Melanoplus femurrubrum.

Figure 41. Six day juvenile.

Figure 42. Seven day juvenile.

Figure 43. Eight day juvenile.

Figure 44. Anterior end of nine day juvenile (total body length, 1.5 mm).

Figure 45. Anterior end of eleven day juvenile (total body length, 2.5 mm).

Figure 46. Anterior end of twelve day juvenile (total body length, approximately 4.8 mm).

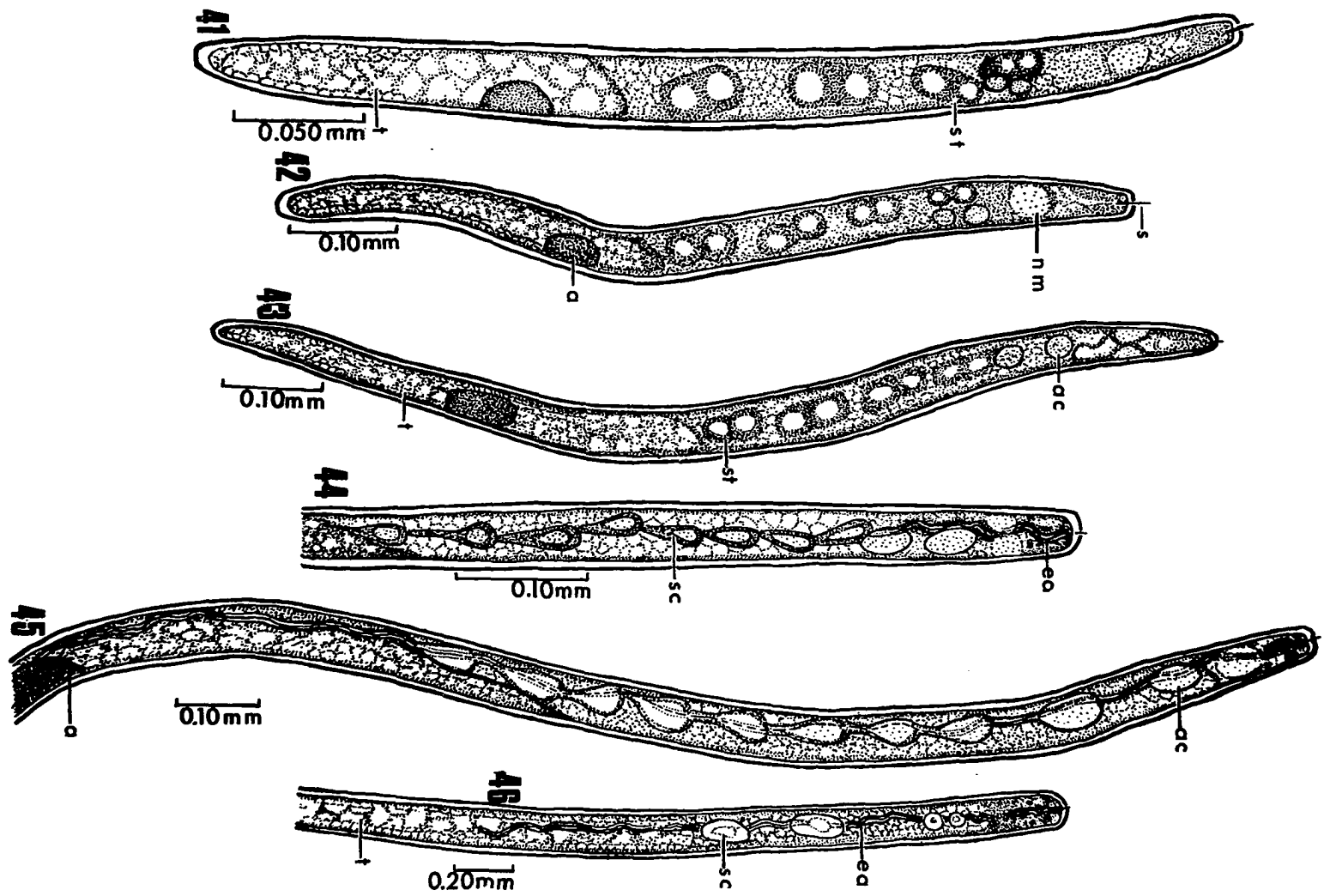


Plate XIII

Figures 47-49. Mermis subnigrescens postparasitic juvenile integument.

Figure 47. Cortical, fibrous, and matrix layers. Approximately 7,000X.

Figure 48. Matrix layer, basal lamella, and hypodermal layer. Approximately 20,000X.

Figure 49. Basal lamella and hypodermis. Approximately 55,000X.

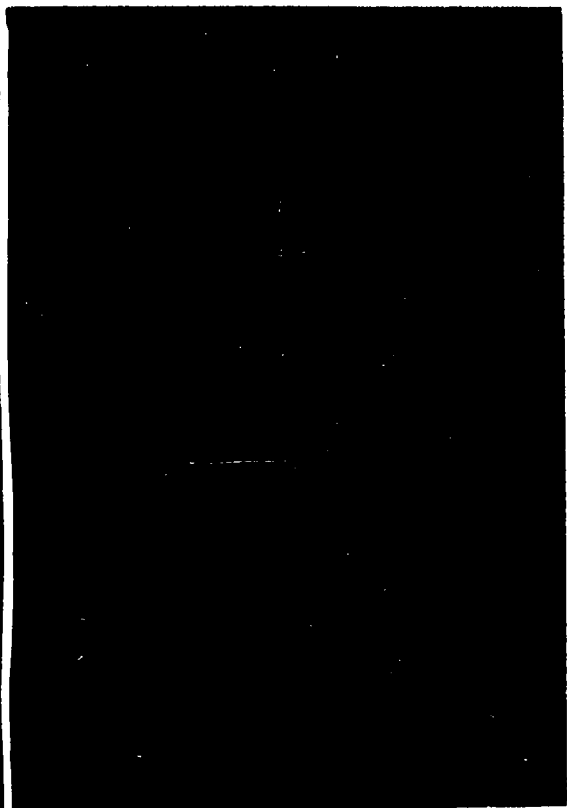
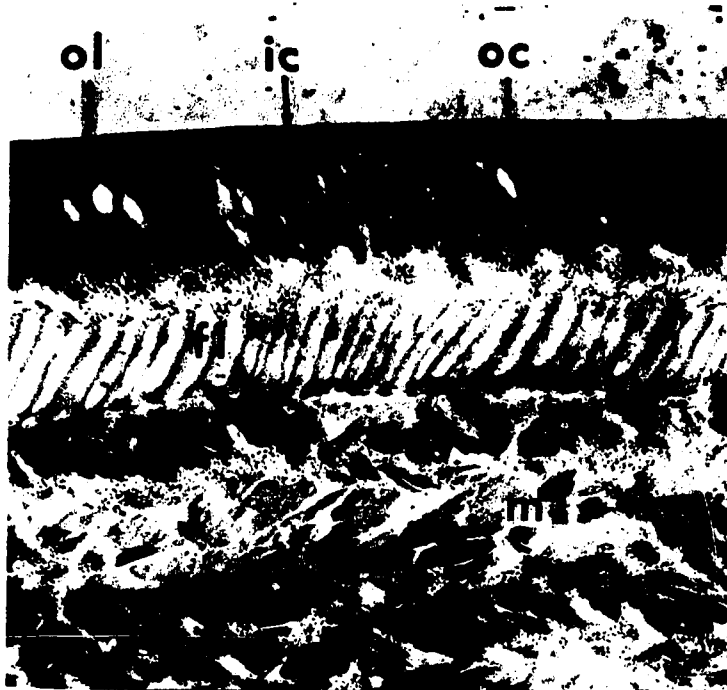


Plate XIV

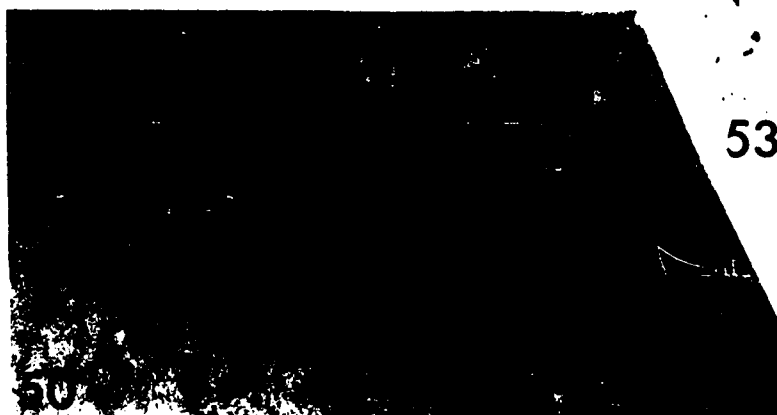
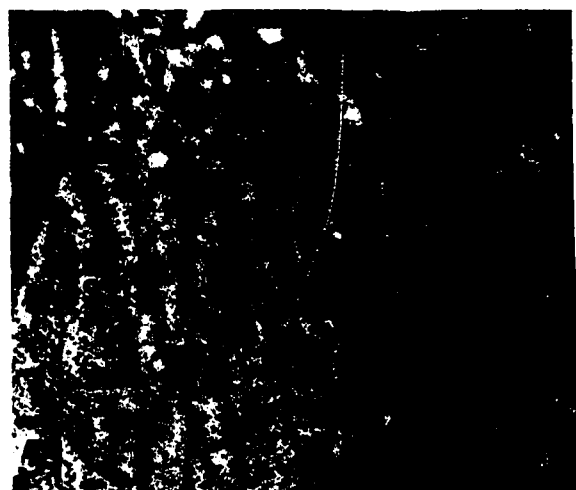
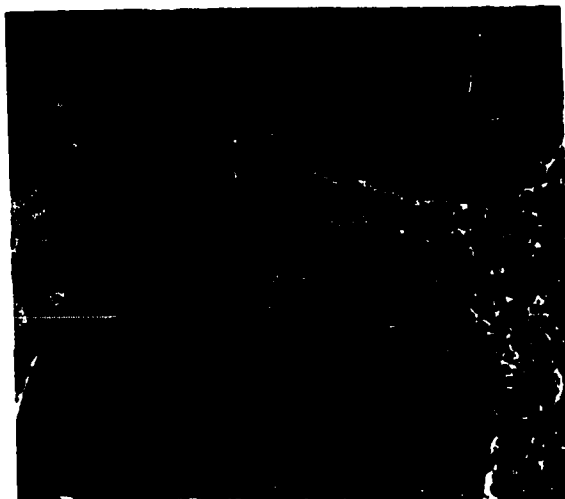
Figures 50-53. Mermis subnigrescens postparasitic juvenile hypodermal layer, body wall musculature, trophosomal spheres, and esophagus.

Figure 50. Cross section through the hypodermal layer. Approximately 12,000X.

Figure 51. Cross section through body wall musculature showing "fast" and "slow" muscle and sarcoplasmic reticulum. Approximately 9,500X.

Figure 52. Trophosomal spheres and glycogen rosettes. Approximately 32,000X.

Figure 53. Cross section through esophageal apparatus. Approximately 12,000X.



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